OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0069005260000

Legal Name*: Southern Research Institute
Department:
Division: Drug Discovery
Street1*: 2000 Ninth Ave. South
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 35205-5305

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	(b) (3) (A), (b) (6)		(b) (3) (A), (b) (6)	

Position/Title: Senior Contract Administrator
Street1*: 2000 Ninth Ave. South
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 35205-5305
Phone Number*: (b) (3) (A), (b) Fax Number: Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Medicinal Chemistry and Lead Development Core - SR

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Southern Research Institute
Duns Number:	0069005260000
Street1*:	2000 Ninth Ave. South
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	35205-5305
Project/Performance Site Congressional District*:	AL-007

Additional Location(s)   File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	MCLDC-Core_C-Abstract.pdf
8. Project Narrative*	
9. Bibliography & References Cited	MCLDC-Core_C-References.pdf
10.Facilities & Other Resources	Med_Chem_Core_Facilities_and_Other_Resources.pdf
11.Equipment	Med_Chem_Core_Major_Equipment.pdf

The Medicinal Chemistry and Lead Development Core (MCLDC) is a key component of the Antiviral Drug Discovery and Development Center (AD3C) that will contribute to the goals of the overall Center of Excellence for Translational Research (CETR) program to develop new replication inhibitors and other broad-based therapeutics for the treatment of emerging pathogens through its interaction with each of the individual projects and cores. The MCLDC will provide a) synthetic chemistry, b) structure-activity relationship (SAR) data and analysis, c) lead optimization chemistry, d) bioanalytical assays, e) structural biology and computational support, and d) compound storage and data management. In this role, the MCLDC, in conjunction with the Assay Core, will be the central focus of the translational research component of the program. As such, the MCLDC will work closely with the Assay Core and each of the Research Project teams. As new chemical entities are designed and synthesized during the lead generation and optimization processes, the Assay Core will test these analogs in SAR-driving assays. Testing of compounds for drug-like properties such as microsomal stability and solubility; and *in vitro* Absorption, Distribution, Metabolism and Excretion (ADME) will be an integral part of SAR studies from the early developmental phase. Appropriate lead compounds will be provided to the various Research Project teams for advanced studies including efficacy, mechanism of action, and other experiments. The resulting data on lead compounds from the Assay Core and Research Projects will then be analyzed by the MCLDC to drive the iterative lead optimization and *in vivo* PK studies to completion, resulting in optimized leads with drug-like properties for animal studies. The lead candidates will have met the go/no go decision criteria set for activity, potency and drug-like properties. The goal for the end result of this process will be identification of novel optimized lead molecules that are appropriate for IND applications. The MCLDC will incorporate key personnel of other Cores and Research Project teams into the prioritization and decision–making procedures.

The Chemistry Department within SR's Drug Discovery Division (DDS) houses a team of chemists with extensive experience in both medicinal chemistry and synthetic organic chemistry. Comprised of a director, three senior investigators, one senior computational investigator, one senior bioanalytical investigator, one structural biology investigator, twelve bench chemists, seven post-doctoral fellows, and two associates, current research and drug discovery programs within the Department cover a large range of various disease areas including cancer, antivirals and emerging pathogens, Parkinson's and Alzheimer's disease, ALS, diabetes, and psychiatric disorders. One of the Chemistry Department's main goals is to identify and bring forth compounds that can be classified as potential preclinical candidates and then collaborate with partners to advance these compounds into human clinical trials. Additionally, compounds are used as tools to investigate novel targets. Several existing collaborative relationships exist between the Department and external partners, such as universities, research centers, and the National Institutes of Health (NIH). The Department encompasses various disciplines in addition to synthetic chemistry, including structural biology and protein crystallography, computational chemistry, bioanalytical, high-throughput synthesis and purification capabilities, as well as informatics tools to process and track data. [redacted] (b) (3) (A), (b) (4) (b) (3) (A), (b) (4) (b) (3) (A), (b) (4) The Department houses 18 fully-equipped chemistry laboratories, as well as one laboratory for microwave-assisted synthesis, one laboratory for parallel synthesis, one laboratory for a walkup LC-MS, and one laboratory for a walkup NMR. Several networked 3D molecular graphics facilities with state-of-the-art molecular modeling hardware and software, informatics software, a protein X-ray Crystallography Laboratory and associated crystallographic software and workstations, several protein crystallization laboratories, two walk-in cold rooms, a notebook/records archive, three Repository laboratories for long-term sample storage, and a glassware washing laboratory, all of which are described below. In addition, there are sufficient offices proximate to the laboratory space to accommodate all senior staff, other offices dedicated to specialized project uses, general conference and meeting rooms, and ample storage space. Other units of DDS (e.g., the Biochemistry and Molecular Biology Department) have facilities reciprocally available to the Chemistry Department as needed. As an aggregate, SR has superb facilities for the design, synthesis, isolation, purification, identification, characterization, scale-up, modeling, and assay of synthetic compounds.

Individual chemistry laboratories are each stocked with all standard equipment and apparatus, including glassware (flasks, still heads, condensers of all types, manifolds, traps, dessicators, drying pistols, thermometers, and separatory, filtering, and addition funnels, etc.), vacuum pumps and gauges, rotary evaporators, rheostats, hot plates, heating mantles, fume hoods (one per person), inert gases and gas handling equipment, gravity and flash chromatography columns, sonicators, balances, melting point apparatus, magnetic and mechanical stirrers, ovens, UV lamps, TLC and preparative layer developing chambers, and explosion-proof refrigerator-freezers.

[redacted] (b) (3) (A), (b) (4), (b) (6)

(b) (3) (A), (b) (4), (b) (6)

[redacted]

Common standard equipment: The Chemistry Department maintains other, more specialized synthetic apparatus that is available to all projects, including: an ozone generator, centrifuges, freeze-dryers, freeze-drying condenser, programmable hot plates, refrigerated water-bath coolers and cold-plates, Immersion Cooler (range 50°C to -150°C) with temperature controller, two low pressure Parr shaker hydrogenators, one custom portable medium-scale atmospheric hydrogenators, large-scale atmospheric hydrogenator, Soxhlet and liquid-liquid extractors, vacuum ovens, ultralow temperature freezers, two OptiMelt Automatic Melting Point System model MPA100 with printer from Stanford Research Systems which is capable of a digital movie of each melt, Rayonet Chamber UV photochemical reactor complete with 16 RPR-3500A lamps and safety shield Model RPR-100, and high pressure reactors. Other equipment available for walk-up use by the chemists are:

- ***Automated Purification Systems.*** The department has eight automated purification systems to be used by chemists in their day-to-day operations:
  - Isolera automated flash purification system with a variable wavelength detector
  - Biotage SP4 HPFC System EXPs with a variable wavelength detector, capable of purifying four samples concurrently
  - Two Teledyne ISCO Combiflash Rf200 systems with a variable wavelength detector
  - One Teledyne ISCO Combiflash Rf200 system with 4x modules that enable automated four column sequential runs equipped with a variable wavelength detector.
  - Two Teledyne ISCO Combiflash Rf200 with 4x modules is equipped with a variable wavelength detector and Evaporative Light Scattering Detector (ELSD) module for compounds with non-UV chromophores.
  - A Yamazan W-prep 2XY dual channel system, capable of running two columns simultaneously
- ***HPLC System.*** One Hewlett-Packard 1100 Series System which includes online vacuum degassers, quaternary pumps, two auto-samplers, one manual injector, three thermostatted column compartments, two diode-array detector and a variable wavelength detector. The HP Chemstation controls the systems and evaluates the data.
- ***Preparative HPLC System.*** A Shimadzu automated preparative HPLC system including 2 solvent selection valves, an auto sampler, photodiode array detector and fraction collector that are computer monitored and operated. This system has the capability of running binary solvent gradients at a flow range of 0.1 to 150 mL/min. Multiple unattended automated runs are possible with this system and the accompanying software package.
- ***LC-MS System.*** An Agilent 6140 LC/MS single quad high throughput bundle featuring a 1200 series LC with RapidRes capability (can handle up to 600 bar pressure). Included with the 1200 series LC is a diode array detector and a 1200 series high performance autosampler. The 6140 single quad MS is equipped with a multimode ESI/APCI source. The setup is operated by Agilent's ChemStation software which is coupled with their Easy Access software to readily allow hands on operation by the chemists in the department.
- ***NMR Spectrometer.*** A Varian 400 MHz Nuclear Magnetic Resonance (NMR) Spectrometer, a 400 MHz NMR with a 4 nuclei probe for ^{1}H, ^{13}C, ^{19}F, and ^{31}P and is equipped with a variable temperature unit and sample changer is solely for walk-up purposes. A second instrument of this type is also available in the BDD Laboratory (see below).

(b) (3) (A), (b) (4)

[redacted]

(b) (3) (A), (b) (4)

[redacted]

(b) (3) (A), (b) (4), (b) (6)

[redacted]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Ashish Middle Name K Last Name*: Pathak Suffix:

Position/Title*:	Advanced Scientist
Organization Name*:	Southern Research
Department:	Chemistry
Division:	Drug Discovery
Street1*:	2000 9th Ave S
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	35205-5305

Phone Number*: 251-581-2542 Fax Number:

E-Mail*: apathak@southernresearch.org

Credential, e.g., agency login (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Core Lead

Degree Type: Ph.D. Degree Year: 1993

Attach Biographical Sketch*: File Name: Biosketch_Pathak_Ashish.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name (b) (3) (A), Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: (b) (3) (A), (b) (6)
Organization Name*: Southern Research
Department:
Division: Drug Discovery
Street1*: (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 35205-5305

Phone Number*: (b) (3) (A), (b) (6) Fax Number:
E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Core Co-Lead

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b) (6).pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name (b) (3) (A), (b) (6) Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: (b) (3) (A), (b) (6)
Organization Name*: Southern Research
Department: Chemistry Department
Division: Drug Discovery
Street1*: (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 35205-5305

Phone Number*: (b) (3) (A), (b) (6) Fax Number:
E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Core Co-Lead

Degree Type: Ph.D. Degree Year: 1988

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b) (6).pdf
Attach Current & Pending Support: File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak	Ph.D	Core Lead		[redacted] (b) (6)			33,133.00	14,148.00	47,281.00
2.		[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Co-Project Lead		[redacted]			16,633.00	7,102.00	23,735.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Co-Project Lead		[redacted]			8,362.00	3,570.00	11,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **82,948.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	[redacted] (b) (6)			100,602.00	42,957.00	143,559.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
8	Chemists	[redacted]			352,430.00	150,487.00	502,917.00
2	Scientists	[redacted]			37,927.00	16,195.00	54,122.00
1	Div Proj Mgr	[redacted]			11,300.00	4,825.00	16,125.00
14	**Total Number Other Personnel**					**Total Other Personnel**	**716,723.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**799,671.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	182,568.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Charges	32,055.00
Total Other Direct Costs	**214,623.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,019,532.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. OH - Salaries + Benefits	120.0	799,671.00	959,605.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	1,979,137.00	395,827.00
3. FCCM - OH Base	7.3	799,671.00	58,376.00
4. FCCM - G&A Base	0.1	1,979,137.00	1,979.00
		Total Indirect Costs	**1,415,787.00**

Cognizant Federal Agency DHHS / Darryl Mayes / 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**2,435,319.00**

J. Fee	**Funds Requested ($)***
	0.00

K. Total Costs and Fee	**Funds Requested ($)***
	2,435,319.00

L. Budget Justification* File Name:
Med_Chem_Core_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak	Ph.D	Core Lead		(b) (6) [redacted]			33,133.00	14,148.00	47,281.00
2.		[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Co-Project Lead		[redacted]			24,995.00	10,673.00	35,668.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Co-Project Lead		[redacted]			8,362.00	3,570.00	11,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **94,881.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
4	Post Doctoral Associates	(b) (6) [redacted]			147,551.00	63,005.00	210,556.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
8	Chemists	[redacted]			358,676.00	153,154.00	511,830.00
2	Scientists	[redacted]			37,952.00	16,206.00	54,158.00
1	Div Proj Mgr	[redacted]			7,533.00	3,217.00	10,750.00
15	**Total Number Other Personnel**					**Total Other Personnel**	**787,294.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**882,175.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute
Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	197,218.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Charges	42,529.00
Total Other Direct Costs	**239,747.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,127,160.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. OH - Salaries + Benefits	120.0	882,175.00	1,058,610.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	2,185,770.00	437,154.00
3. FCCM - OH Base	7.3	882,175.00	64,399.00
4. FCCM - G&A Base	0.1	2,185,770.00	2,186.00
		Total Indirect Costs	**1,562,349.00**

Cognizant Federal Agency DHHS / Darryl Mayes / 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**2,689,509.00**

J. Fee	**Funds Requested ($)***
	0.00

K. Total Costs and Fee	**Funds Requested ($)***
	2,689,509.00

L. Budget Justification* File Name:
Med_Chem_Core_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak	Ph.D	Core Lead		(b) (6) [redacted]			33,133.00	14,148.00	47,281.00
2.		[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Co-Project Lead		[redacted]			24,995.00	10,673.00	35,668.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Co-Project Lead		[redacted]			8,362.00	3,570.00	11,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **94,881.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
4	Post Doctoral Associates	(b) (6) [redacted]			147,551.00	63,005.00	210,556.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
8	Chemists	[redacted]			358,676.00	153,154.00	511,830.00
2	Scientists	[redacted]			37,952.00	16,206.00	54,158.00
1	Div Proj Mgr	[redacted]			7,533.00	3,217.00	10,750.00
15	**Total Number Other Personnel**					**Total Other Personnel**	**787,294.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**882,175.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute
Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	197,218.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Charges	42,529.00
Total Other Direct Costs	**239,747.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,127,160.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. OH - Salaries + Benefits	120.0	882,175.00	1,058,610.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	2,185,770.00	437,154.00
3. FCCM - OH Base	7.3	882,175.00	64,399.00
4. FCCM - G&A Base	0.1	2,185,770.00	2,186.00
		Total Indirect Costs	**1,562,349.00**

Cognizant Federal Agency DHHS / Darryl Mayes / 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**2,689,509.00**

J. Fee	**Funds Requested ($)***
	0.00

K. Total Costs and Fee	**Funds Requested ($)***
	2,689,509.00

L. Budget Justification* File Name:
Med_Chem_Core_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak	Ph.D	Core Lead		(b) (6) [redacted]			33,133.00	14,148.00	47,281.00
2.		[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Co-Project Lead		[redacted]			24,995.00	10,673.00	35,668.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Co-Project Lead		[redacted]			8,362.00	3,570.00	11,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **94,881.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
4	Post Doctoral Associates	(b) (6) [redacted]			147,551.00	63,005.00	210,556.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
8	Chemists	[redacted]			358,676.00	153,154.00	511,830.00
2	Scientists	[redacted]			37,952.00	16,206.00	54,158.00
1	Div Proj Mgr	[redacted]			7,533.00	3,217.00	10,750.00
15	**Total Number Other Personnel**					**Total Other Personnel**	**787,294.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**882,175.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute
Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	197,218.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Charges	42,529.00
Total Other Direct Costs	**239,747.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,127,160.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. OH - Salaries + Benefits	120.0	882,175.00	1,058,610.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	2,185,770.00	437,154.00
3. FCCM - OH Base	7.3	882,175.00	64,399.00
4. FCCM - G&A Base	0.1	2,185,770.00	2,186.00
		Total Indirect Costs	**1,562,349.00**

Cognizant Federal Agency DHHS / Darryl Mayes / 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**2,689,509.00**

J. Fee	**Funds Requested ($)***
	0.00

K. Total Costs and Fee	**Funds Requested ($)***
	2,689,509.00

L. Budget Justification* File Name:
Med_Chem_Core_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak	Ph.D	Core Lead		(b) (6) [redacted]			33,133.00	14,148.00	47,281.00
2.		[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Co-Project Lead		[redacted]			24,995.00	10,673.00	35,668.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Co-Project Lead		[redacted]			8,362.00	3,570.00	11,932.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **94,881.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
4	Post Doctoral Associates	(b) (6) [redacted]			147,551.00	63,005.00	210,556.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
8	Chemists	[redacted]			358,676.00	153,154.00	511,830.00
2	Scientists	[redacted]			37,952.00	16,206.00	54,158.00
1	Div Proj Mgr	[redacted]			7,533.00	3,217.00	10,750.00
15	**Total Number Other Personnel**					**Total Other Personnel**	**787,294.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**882,175.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	197,218.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Charges	42,529.00
Total Other Direct Costs	**239,747.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,127,160.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
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2. G&A - Salaries + Benefits + OH + ODC	20.0	2,185,770.00	437,154.00
3. FCCM - OH Base	7.3	882,175.00	64,399.00
4. FCCM - G&A Base	0.1	2,185,770.00	2,186.00
		Total Indirect Costs	**1,562,349.00**

Cognizant Federal Agency DHHS / Darryl Mayes / 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**2,689,509.00**

J. Fee	**Funds Requested ($)***
	0.00

K. Total Costs and Fee	**Funds Requested ($)***
	2,689,509.00

L. Budget Justification* File Name:
Med_Chem_Core_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Key Personnel:

Ashish Pathak, Ph.D. *(Y1-Y5: (b) (6) cal. months).* Dr. Pathak will serve as Core Lead for the Medicinal Chemistry Core. Dr. Pathak is Advanced Research Scientist in the Chemistry Department in the Drug Discovery Division of SR and holds an Adjunct Professor appointment in the Chemistry Department at the University of Alabama at Birmingham (UAB). He has over 27 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective and vaccine adjuvant drug design. He has established himself as an independent researcher at Southern Research (SR) and previously led research groups in the Department of Chemistry at Western Illinois University as an Assistant Professor before joining SR. Currently, he manages the high-throughput parallel synthesis group. He is actively involved in research work in drug discovery and is leading medicinal chemist efforts on several externally and in-house funded drug discovery programs. He has extensive experience in all aspects of a medicinal chemistry program, which extends across the spectrum of early lead discovery to lead optimization. He has been PI of two R21 NIH-funded programs in the area of viral vaccine adjuvant discovery. He serves as the current Medicinal Chemistry and Lead Development Core Project Leader of the Antiviral Drug Discovery and Development Center (AD3C) funded by CETR. He is also leading several drug discovery programs in cancer and anti-infectives. In collaboration with the project PIs and other Co-Investigators in the Center, Dr. Pathak will oversee all aspects of the medicinal chemistry effort, including synthetic target selection, target design, synthesis, and the planning of synthetic routes, compound characterization, structure-activity relationship analysis and interpretation of biological results, cheminformatics and molecular modeling, report, manuscript, and patent application preparation and overall project management of lead optimization chemistry. He will also provide experimental design guidance for ADME and *in vivo* pharmacokinetic (PK) studies.

(b) (3) (A), (b) (6)**, Ph.D.** *(Y1: (b) (6) cal. months; Y2-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6) will serve as Co-Core Lead for the Medicinal Chemistry and Lead Development Core. (b) (3) (A), (b) (6) is extensively trained as an organic and medicinal chemist, and an accomplished scientific leader with nearly (b) (3) (A), (b) (6) of drug discovery and management experience. (b) (3) (A), (b) (6) will be responsible for directing all medicinal chemistry and PK efforts for any drug discovery effort of which go/no go decision trees and timelines will facilitate progress and success. (b) (3) (b) (3) (A), (b) (6) has managed more than 30 different drug discovery programs covering at least ten different therapeutic areas. (b) (3) (A), (b) has been responsible for multi-disciplinary project teams at sites worldwide from which clinical candidates have been identified in areas such as Alzheimer's disease, anxiety and inflammation. (b) (3) (A), (b) (6) (b) (3) (A), (b) (6) has been invited to speak world-wide on (b) (3) (A), (b) academic drug discovery research, and (b) (3) (A), has consulted for various drug discovery efforts. (b) (3) (A), (b) has been an active member of NIH study sections for several years and has served as a faculty sponsor and mentor to graduate and undergraduate students. (b) (3) (A), (b) has been an advisor to scientific meetings and a reviewer for several journals, and (b) (3) (A), currently holds an adjunct professor position in the (b) (3) (A), (b) (6)

(b) (3) (A), (b) (6)**, Ph.D.** *(Y1-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6) will also serve as Co-Core Lead for the Medicinal Chemistry and Lead Development Core. (b) (3) (A), (b) (6) is the (b) (3) (A), (b) (6) He has more than (b) (3) (A), (b) (6) years of drug discovery and management experience, serving in a range of capacities, from bench medicinal chemist to leading drug discovery programs at major pharmaceutical (b) (3) (A), (b) (6) as well as smaller biotech and venture-backed organizations. As a medicinal chemist, he is not restricted to a single therapeutic area and relies on collaborations with fellow investigators for developing novel targets and approaches and for using various methodologies such as HTS-driven or virtual screening to identify hits or leads that can be optimized and advanced into animal models. He and the Chemistry Department at SR cumulatively have the capabilities [i.e., assay development, hit identification (screening), and lead development and optimization, utilizing medicinal, computational and analytical chemistry, as well as *in vitro* and *in vivo* PK] needed to support the goal of developing new therapeutics and moving compounds into clinical development.

Other Personnel:

(b) (3) (A), (b) (6)**, M.S., P.M.P.** *(Y1: (b) (6) cal. months; Y2-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6), Divisional Project Manager, has over (b) (3) (A), (b) (6) years of experience in coordinating and managing research projects, and prior to that, (b) (3) (A), was a Senior Biologist in the (b) (3) (A), (b) (6), where (b) (3) (A), contributed considerably to assay adaptation to high throughput format. (b) (3) (A), (b) (6) will work with Dr. Pathak, the other project leaders and the chemistry team to ensure timely and efficient transfer of project information, resources and data between internal and external project team members. (b) (3) (A), (b) (6) will also track sample transfer between the departments and institutes and manage the testing of samples to assure the deliverables and milestones identified within the project timeline are met.

Medicinal Chemistry Group:

(b) (3) (A), (b) (6)**, Ph.D.** *(Y1-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6) will support Dr. Pathak in running the Core with his antiviral drug discovery expertise as a Senior Medicinal Chemist. (b) (3) (A), (b) (6) studied synthesis of modified cyclic/acyclic nucleoside analogs as antitumor, antiviral and anti-tuberculosis agents. He has more than (b) (3) (A), (b) (6) years of extensive research experience in the area of synthesis of potential anticancer, antiviral, antibiotic nucleoside chemistry and small-molecule drug discovery. With his broad synthetic background and his experience with solid-, solution-, and liquid- phase methodologies, employing both robotic and manual protocols, he has been the lead chemist on several NIH-funded grant projects.

(b) (3) (A), (b) (6)**, Ph.D. and** (b) (3) (A), (b) (6)**, Ph.D** *(Y1-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6), Senior Chemists in the Department of Chemistry, will provide chemistry services and are members of the currently funded CETR-funded AD3C Medicinal Chemistry and Lead Development Core. They will be directly responsible for day-to-day synthetic activities in the laboratory. They will perform compound synthesis, compound scale-up, and library research, and will assist in target selection, analog design, synthetic methodology planning, compound characterization, data analyses, cheminformatics and molecular modeling. Each of these chemists has several years of post-doctoral research experience in the design and preparation of compounds of many types, and analyzing biological data for structure-activity relationships. Each will devote 100% effort to this project over the duration of the grant award in performing hit to lead chemistry on active series against various viruses (Projects 1-4) and scale-up synthesis of lead molecules for animal studies.

(b) (3) (A), (b) (6)**, Ph.D.** *(Y1-Y5: (b) (6) cal. months)*, (b) (3) (A), (b) (6)**, Ph.D.** *(Y2-Y5: (b) (6) cal. months),* and (b) (3) (A), (b) (6)**, Ph.D.** *(Y1-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6), Postdoctoral Chemistry Researchers, are experienced medicinal chemists that have worked on hit-to-lead and lead optimization projects in the area of anti-infectives. (b) (3) (A), (b) (6) are chemists working in the current AD3C funded through CETR. They will be responsible for the synthesis of new compounds and integration of the results obtained as the program proceeds. They will develop new potent derivatives of the initial lead series with improved ADME properties.

(b) (3) (A), (b) (6)**, M.S.** *(Y1-Y5: (b) (6) cal. months)*, Research Chemist, is an experienced multistep organic chemist with a vast knowledge of many types of organic reactions involving both small and large scale synthesis. He also has experience in the pharmaceutical industry (b) (3) (A), (b) (6) years synthesis and drug design), chemical industry (b) (3) (A), (b) (6) years synthesis and route design), and process chemistry (b) (3) (A), (b) (6) years synthesis). He will serve as a Chemist for this proposal and will be responsible for the synthesis of compounds and data analysis. He will also be responsible to synthesize intermediates and large scale synthesis.

(b) (3) (A), (b) (6)**, M.S.** *(Y1-Y5: (b) (6) cal. months)*, Chemist, has a broad background in chemistry with specialized interests in analytical and organic chemistry. He has (b) (3) (A), (b) (6) years of experience in an industrial setting including time as a QC analyst and as a process chemist in addition to his graduate studies in synthetic organic chemistry in the fields of hereditary angioedema (HAE), breast and prostate cancers, HIV-1, and flavivirus infectivity. He will serve as a Chemist for this proposal and will be responsible for the synthesis of compounds and data analysis. He will also be responsible to synthesize intermediates and large scale synthesis. He is one of the chemists working in the current AD3C funded through CETR.

Bioanalytical Group:

(b) (3) (A), (b) (6) **Ph.D.** *(Y1-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6), Senior Scientist, is head of the analytical laboratory at SR and will be responsible for all of the *in vitro* and *in vivo* ADME studies that will be used to assess the compounds metabolic stability, solubility and permeability. (b) (3) (A), (b) (6) has more than (b) (3) (A), (b) (6) years of extensive research, bioanalytical, and DMPK experience at SR and the biotech industry performing studies to support drug discovery and clinical development programs.

(b) (3) (A), (b) (6)**, Ph.D.** *(Y1: (b) (6) cal. months; Y2-Y5: (b) (6) cal. months).* Under the direction of (b) (3) (A), (b) (6), Associate Research Chemist, will conduct the *in vitro* ADME studies to determine plasma concentrations of the best compounds. This includes formulation work, dosing and sample analysis. (b) (3) (A), (b) (6) has (b) (3) (A), (b) (6) years of experience in Drug Discovery and Toxicology, and is skilled in analytical instrumentation and method development. His focus will be to determine plasma concentrations of the best compounds, which includes formulation work, dosing and sample analysis at SR's state of the art bioanalytical laboratory. (b) (3) (A), (b) (6) has (b) (3) (A), (b) years of experience in Drug Discovery and Toxicology, and is skilled in analytical instrumentation and method development.

Chemist (Bioanalytical) (To be hired) *(Y1-Y5: 12 cal. months).* A BS/MS analytical chemist will be hired with laboratory experience to carryout ADME and other analytical experiments required to support medicinal chemistry.

Structural Biology and Computational Chemistry Group:

(b) (3) (A), (b) (6)***, Ph.D.*** *(Y1: (b) (6) cal. months; Y2-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6), Senior Scientist, has over (b) (3) years of experience in protein sciences including protein expression, protein purification, functional characterization, protein identification and structure determination. He holds a Master's of Science in Biochemistry and a Ph.D. in X-ray crystallography. His lab currently focuses on structure-based drug discovery by providing the details of protein-compound atomic structure to develop new compounds. For this project, (b) (3) (b) (3) (A), will provide variable efforts once the viral proteins which the anti-viral compounds are targeting are identified. His laboratory will provide support such as cloning, expression and purification of target proteins, characterization of protein-compound binding, and determination of protein-compound structures. The goal of his laboratory in this project is to determine the atomic structure of protein-compound complexes and provide the details to the chemists to assist with the design of potent compounds.

(b) (3) (A), (b) (6)***, Ph.D.*** *(Y1: (b) (6) cal. months, Y2-Y5: (b) (6) cal. months).* (b) (3) (A), (b) (6), Postdoctoral Chemistry Researcher, will provide computational chemistry support including conducting modeling experiments which will be used to prioritize and design compounds for synthesis. In addition, he will assist with developing models and algorithms for structure-activity and PK properties.

Compound Management Group:

(b) (3) (A), (b) (6) *(Y1-Y5: (b) (6) cal. months) (Chemist, Supervisor of Compound Management, Chemistry Department)* has extensive experience in managing in-house synthesized compounds and commercial libraries. He will provide integrated informatics support including compound tracking, data capture, and data storage, backup, and retrieval. He is in charge of maintaining our in-house Dotmatics registration database, which is used extensively in this program to assign a unique identifier to each compound synthesized or commercially-acquired. This identifier number is used throughout the Center to track compounds and any associated data. He is also responsible for sample preparation and distribution to the Assay Core and research project sites.

Other (Non-Labor) Direct Costs *(Y1: $219,861; Y2-Y5: $244,985/y)*

Supplies (*Y1: $170,623; Y2-Y5: $186,747/y).* This budget includes reagents and solvents for six synthetic chemist FTE's in Y1 and seven FTE's chemists in Y2-Y5, estimates based on the historic average cost, catalog pricing from SR-approved vendors (many of whom provide Alabama state contracted rates), historical patterns of laboratory usage, and the PI's past experiences in lead optimization chemistry programs. This budget covers starting materials, specialized reagents, solvents, chromatography supplies, resins and solid-phase synthesis supports, glassware, plastic ware, Eppendorf and other tubes capable of holding larger liquid volumes and other disposables, as well as spectroscopy and compound characterization expenses. It also includes ADME supplies for PK profiling of compounds and potential drug candidates emerging from the lead optimization program. The execution of the various *in vitro* ADME studies, such as solubility, permeability and microsomal stability, requires the purchase of various disposables needed to perform these studies, including microtiter plates, reagents, solvents and other miscellaneous consumables which are essential to the proposed ADME studies. It also include supplies for the structural biology group to carryout protein purification and crystallization efforts.

Travel Expenses *(Y1-Y5: $5,238/y).* Funds are allocated for the Project Leader and Co-Project Leaders to attend the required NIAID CETR Program Meeting.

Other Charges *(Y1: 44,000; Y2-Y5: $53,000/y).*

***In vivo* PK Studies** *(Y1-Y3: $32,000/Y, Y4-Y5: 39,000/Y)*. The PK Studies will be out sourced to Pharmaron Inc., a Contract Research Organization.

Waste Disposal – *(Y1: $12,000, Y2-Y5: $14,000/Y)* has been allocated for the disposal of hazardous chemical and other wastes generated in the execution of the experiments.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		462,472.00
Section B, Other Personnel		3,865,899.00
Total Number Other Personnel	74	
Total Salary, Wages and Fringe Benefits (A+B)		4,328,371.00
Section C, Equipment		0.00
Section D, Travel		26,190.00
1. Domestic	26,190.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		1,173,611.00
1. Materials and Supplies	971,440.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	202,171.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		5,528,172.00
Section H, Indirect Costs		7,665,183.00
Section I, Total Direct and Indirect Costs (G + H)		13,193,355.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		13,193,355.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	MCLDC-Core_C-Specific_Aims.pdf
3. Research Strategy*	MCLDC-Core_C-Research_Strategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	3-14-2018_MCLDC_-_Vertebrate_Animals_Section.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Core_C.pdf
9. Letters of Support	
10. Resource Sharing Plan(s)	MCLDC-Core_C-Resource_Sharing_Plan.pdf
11. Authentication of Key Biological and/or Chemical Resources	MCLDC_AUTHENTICATION_OF_KEY_RESOURCES.pdf
Appendix	
12. Appendix	

The Medicinal Chemistry and Lead Development Core (MCLDC), in conjunction with the Assay Core, will be the central focus of the translational research component of the Center of Excellence for Translational Research (CETR) Antiviral Drug Discovery and Development (AD3C) program from which leads will be generated to develop drug candidates with optimized biological and biophysical properties. The MCLDC will provide hit identification, hit-to-lead analysis, lead optimization chemistry, computational and structural biology analysis, pharmacokinetic (PK) support, and informatics tools to support the AD3C's goal of developing new broad-spectrum therapeutics for (re-)emerging infections of coronaviruses, alphaviruses, flaviviruses and influenza. Evaluation of compounds in a panel of *in vitro* ADME (Absorption, Distribution, Metabolism and Excretion) assays, such as microsomal stability and solubility for drug-like properties, will be an integral part of structure-activity relationship (SAR) studies from the early developmental phase. Lead compounds will be provided to the various Research Project teams for testing in secondary biological assays from which the resulting data will be analyzed by the MCLDC and used in an iterative process of compound optimization. Frequent interactions of the MCLDC with each of the Research Projects and Cores to facilitate progression of the core goals have been carefully planned and are outlined in detail in the proposal.

SPECIFIC AIMS

Aim 1: ***Provide lead generation and optimization through medicinal chemistry efforts, SAR studies, structural biology and computational chemistry analyses, and ADME and pharmacokinetic (PK) data to support compound progression into in vivo antiviral efficacy models at Research Project sites.***

(b) (4)

[redacted]

Aim 2: ***Provide integrated informatics support which includes compound tracking, as well as data capture, analysis, storage, backup and retrieval.***

Informatics support is an integral part of any drug discovery process, especially for a drug discovery program of this size since a large number of compounds will be synthesized and tested in variety of assays. Careful tracking of compounds submitted to the Assay Core for testing in the primary SAR screen as well as coordination of data generated from the Research Project sites will be important. Compounds coming from Emory Institute for Drug Development (EIDD) will also be distributed to the corresponding Research Project sites via SR's compound management group. SR will use a secured, access-limited, [redacted] (b) (3) (A), (b) (4)

INTRODUCTION

The Medicinal Chemistry and Lead Development Core (MCLDC) of Southern Research (SR) will provide hit-to-lead analysis, lead optimization chemistry, computational and structural biology analysis, PK support, and informatics tools to support the AD3C's goal of developing new broad-spectrum therapeutics for (re-)emerging infections of coronaviruses, alphaviruses, flaviviruses and influenza. SR, a not-for-profit research organization, began a highly successful translational drug discovery program in 1954 with the goal of identifying novel or improved agents to target the treatment of widespread chronic and infectious diseases, and to promote transition of these agents into the clinic. This required and led to the establishment of medicinal chemistry-centered multidisciplinary teams to methodically evaluate all aspects of candidate drug molecules in-house, which is directly applicable to this proposal. SR has discovered seven FDA approved drugs from discovery programs funded almost exclusively through NIH grants and other government contracts. SR regularly collaborates with the University of Alabama at Birmingham, for instance, through its partnership with the current AD3C CETR, in addition to the Alabama Drug Discovery Alliance (ADDA), which aims to support the discovery and development of new targets and the identification and validation of novel small molecules that address unmet medical needs.

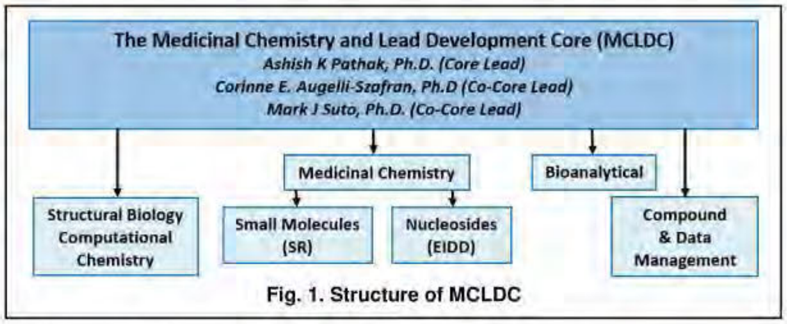
Relationship of the Core to Project Theme and to Other Projects and Cores. The MCLDC, in conjunction with the Assay Core (designated as the Screening Core in the current CETR), will be the central focus of the translational research component of the program where lead compounds will be generated to develop potential drug candidates that possess optimized biological and biophysical properties for the treatment of emerging viral pathogens. Specialized primary screens and structure-activity relationship (SAR)-driving assays are completed by the Assay Core, facilitating a timely turnaround of biological data by the Research Project Leaders. This collaborative interaction is necessary for the MCLDC to then fulfill their role of developing SAR for each project. Through ongoing efforts of the current AD3C CETR, the MCLDC has provided high-throughput screening (HTS) data and analyses, hit-to-lead analysis, structure-activity relationship (SAR) as well as computational, structural biology, and pharmacokinetic (PK) support. As demonstrated in the past, the MCLDC will work very closely and collaboratively with all Research Project and Core personnel. In this CETR application, we will also be working closely with two additional research sites, the University of Texas Medical Branch (UTMB; Project 1, Flaviviruses) and the Emory Institute for Drug Development (EIDD). Together, a comprehensive project plan has been crafted, including lead compound profiling for a particular target, a workflow structure, timelines, key milestones and deliverables, as well as go/no go decision points. Decisions about the specific biological assays to be used to drive the SAR, and the type and extent of optimization appropriate for each given compound or chemical series, are expected to vary according to project and are made in conjunction with Project Leaders and other Key Personnel of the AD3C CETR project team. Lead compounds will be provided to the various Research Project teams for advanced studies, from which the resulting data will be analyzed by the MCLDC in order to drive the iterative drug discovery program to completion. Regularly scheduled discussions among members of the Assay Core and Research Project teams will facilitate the prioritization of MCLDC efforts and decisions.

The team of experts comprising the MCLDC and their coordinated interactions with the various Research Projects and Cores represent a unique approach to drug discovery. The MCLDC offers a centralized comprehensive chemistry and informatics platform, representing an efficient means to service and coordinate the disparate drug discovery activities essential to the various independent biological teams. The MCLDC is made up of an already assembled working group of medicinal, computational and bioanalytical chemists and structural biologists with experience in industry as well as in academia to carry out comprehensive drug discovery efforts to support the Research Projects. We will extensively integrate computational models and experimental PK and ADME data to design compounds with optimized properties early on in the drug discovery continuum, efforts which help to minimize the risk of pursuing scaffolds that might ultimately prove unsuitable for subsequent drug development. SR maintains several state-of-the-art computational suites that will be employed for this purpose. As one example, we will use the (b) (3) (A), (b) (4) to develop physicochemical profiles and design synthetic targets with commensurate properties. Though each virus under study has compounds structurally unique to that pathogen, the MCLDC will be ideally positioned to identify and utilize any common scaffolds that emerge that might suggest broader spectrum activity or common mechanisms of action.

APPROACH

Specific Aims 1 and 2 will be carried out by the MCLDC through the coordination of different groups within the center as shown in **Fig. 1**. The core leaders have several years of experience in managing and executing multi-disciplinary drug discovery projects in academic as well as industrial environments. Most of the medicinal chemistry activities of the MCLDC will be carried out at the SR site with EIDD as a specialized group for nucleoside chemistry and drug development which will work directly with the SR medicinal chemistry team.

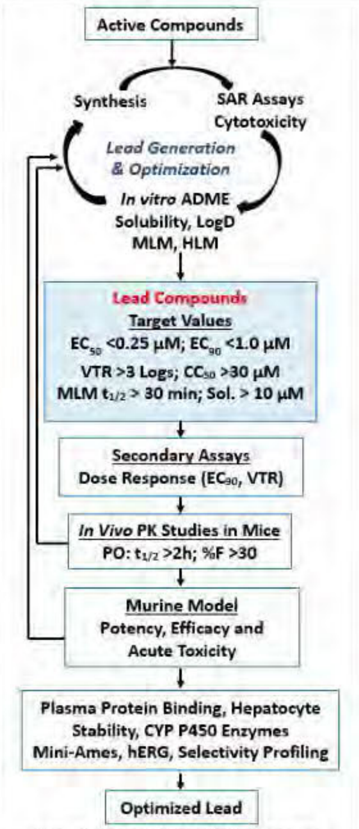
Regular interactions within the group and with Research Project and Core Leaders will be central for go/no-go decisions on a particular compound series to move a program forward. Groups that will support the operations in MCLDC located within the SR Chemistry Department are: a) medicinal chemistry group, b) bioanalytical group, c) structural biology and computational group; and d) compound and data management group.



The Medicinal Chemistry and Lead Development Core (MCLDC)
Ashish K Pathak, Ph.D. (Core Lead)
Corinne E. Augelli-Szafran, Ph.D (Co-Core Lead)
Mark J Suto, Ph.D. (Co-Core Lead)
Medicinal Chemistry
Bioanalytical
Structural Biology Computational Chemistry
Small Molecules (SR)
Nucleosides (EIDD)
Compound & Data Management

Fig. 1. Structure of MCLDC

A. Medicinal Chemistry Group. The medicinal chemistry group is made up of a synthetic group at SR and a nucleoside specialized group EIDD. The team of medicinal chemists at SR and EIDD have far-reaching experience in the synthesis of organic compounds of many different structural classes. These groups will design, synthesize and generate SAR data. Varying the polarity or hydrophobicity, charge state, hydrogen bonding potential, size and other steric parameters will modulate the avidity of a compound for its biological activity and may facilitate a detailed understanding of the pharmacophore and the compound's requirements for binding and activity. The core scaffold may also be modified to examine closely-related templates that may have the potential for improving efficacy or physicochemical properties, such as solubility or metabolism. Thorough lead generation, SAR studies and optimization will be carried out as described in the compound progression pathway (**Fig. 2**), which illustrates the various steps involved to obtain an optimized lead compound for pre-clinical development (as described below).



Active Compounds
Synthesis
SAR Assays Cytotoxicity
Lead Generation & Optimization
In vitro ADME Solubility, LogD MLM, HLM
Lead Compounds
Target Values
EC_{50} <0.25 μM; EC_{90} <1.0 μM
VTR >3 Logs; CC_{50} >30 μM
MLM $t_{1/2}$ > 30 min; Sol. > 10 μM
Secondary Assays
Dose Response (EC_{90}, VTR)
In Vivo PK Studies in Mice
PO: $t_{1/2}$ >2h; %F >30
Murine Model
Potency, Efficacy and Acute Toxicity
Plasma Protein Binding, Hepatocyte Stability, CYP P450 Enzymes Mini-Ames, hERG, Selectivity Profiling
Optimized Lead

Fig. 2. Compound Progression Pathway

Small Molecule Group. This group is located within the SR Chemistry Department and encompasses various disciplines, including synthetic chemistry, structural biology, computational chemistry, bioanalytical, high-throughput synthesis and purification; and informatics tools to process and track data. In-house tools currently exist to design, synthesize, and evaluate all relevant physicochemical and PK properties of small molecules.

(b) (4)

some cases, inactive compounds will also be evaluated for ADME properties to help guide the design process and to understand the effect of chemical modification for solubility or microsomal stability. This iterative process will be carried out until target values (see **Fig. 2**) are achieved. These compounds with desired antiviral activity and acceptable ADME properties will advance to secondary assays at the Research Project sites to provide pathogen-related potency (EC_{90} and VTR) of those compounds. The SR team will work to not only optimize the compound for oral delivery for the viruses under study, but will also optimize compounds to cross the blood brain barrier for the VEEV pathogen program. Hence, additional parameters will need to be evaluated during the design process, such as calculated polar surface area (tPSA = 40–70) and logD = 2–4. *In silico* model predictions of brain permeation will also be utilized during the analog design process.[1] An *in vitro* permeability assay to determine absorption, permeation and transport of compounds into tissues and across cell membranes (MDR1-MDCK or Caco2) will be performed on potential active compounds. P-glycoprotein (PgP) efflux is a significant limitation to brain permeability and thus, it is important to assess PgP efflux early in a series using an *in vitro* PgP assay (P_{app} ≥ 10×10^{-6} cm/s, Efflux ratio ER ≤ 2.5).[2] Compounds selected for SAR studies and lead optimization from the current CETR grant are shown in **Table 1**. Design and synthesis of the new analogs of lead series for different viruses will be based on chemical modifications in an effort to improve activity and drug-

(b) (4)

[redacted]

through the synthesis of compounds, metabolism can be blocked. In addition, advanced compounds will also be tested in a panel of assays for off-targets liabilities. This will be done through testing in a wide range of target classes are including GPCRs, kinases and nuclear receptors at 10 μM. Any compound with activity <50% inhibition will be evaluated further. In addition, compounds will also be tested in the mini-Ames mutagenicity test [mutagenic potential of new drugs in two strains of *Salmonella typhimurium* (TA98 and TA100)] and evaluated for hERG activity (the binding and inhibition of the hERG potassium channel provides critical information on the potential of the compound to cause cardiac risk) (Low: IC_{50} > 10 μM; Moderate: 1 μM < IC_{50} < 10 μM; High: IC_{50} < 1 μM).

Nucleoside Specialized Group: EIDD will provide scientific expertise and a unique collection of 800 ribonucleosides and 5'-phosphate prodrugs for antiviral testing against viruses in all projects. The EIDD compounds were rationally designed to target virally encoded RNA-dependent RNA polymerases (RdRps) which are essential enzymes that are responsible for transcribing viral mRNA and act as a replicase to copy viral genomic RNA. There is no known host cell equivalent, which makes this approach very unique for developing antiviral drugs. Ribonucleoside analogs (as is the case for the natural ribonucleoside substrates) must be anabolized by cellular kinases in three distinct steps to their corresponding 5'-triphosphate (TP) in order to act as competitive, alternative substrate inhibitors of virally encoded RdRps.[4] The compounds are rationally designed as competitive, alternative substrate inhibitors of virally encoded RdRps which will be tested for viruses in all four projects. This process, outlined in **Fig. 4**, will be used for the optimization of ribonucleoside scaffolds. The selection criteria for advancing a ribonucleoside analog through the process is defined as such: *in vitro* potency (extracellular polymerase inhibition) IC_{50} < 5 μM; cellular model of infection EC_{50} < 5 μM; bioavailability > 10%; plasma half life > 1 hr. The process begins with an iterative cycle of analog design, synthesis and biological testing. A unique feature of this cycle is the use of an extracellular, virally-encoded polymerase assay as a primary assessment to determine the intrinsic inhibitory activity of the 5'-TP forms of synthesized ribonucleoside analogs. Such a process circumvents the complicating issues of cellular uptake and anabolism that make it very difficult to interpret cellular replicon or viral infection assays. Cellular infection assays are then carried out with all ribonucleosides and ribonucleoside-5'-phosphate prodrugs where the corresponding 5'-TP demonstrates an IC_{50} < 5 μM. Compounds that show suitable activity in the cellular infection assays (EC_{50} < 5 μM) and have a cellular safety index (SI > 10) will advance into early pharmacological assessments to validate suitability for development studies. Focused libraries will be prepared around active molecules utilizing late-stage glycosylation between modified nucleobases and modified ribofuranoside intermediates. Those ribonucleosides that are inactive in cellular infection assays will be further assessed in cellular anabolism assays to identify the anabolic block that might be responsible for lack of cellular efficacy. The EIDD has established sensitive LC/MS/MS methods for the quantitative determination of intracellular levels of parent ribonucleoside and prodrug conjugates as well as the 5'-monophosphate, 5'-diphosphate, and 5'-TP metabolites in samples from *in vitro* and *in vivo* studies. Prodrug conjugates of the inactive ribonucleosides will be prepared in order to overcome the problematic step to achieve 5'-TP concentrations that are necessary for activity in cellular assays. The vast majority of the compounds in the library have been screened against lymphoid and hepatocyte cell lines and have been found to be non-cytotoxic.

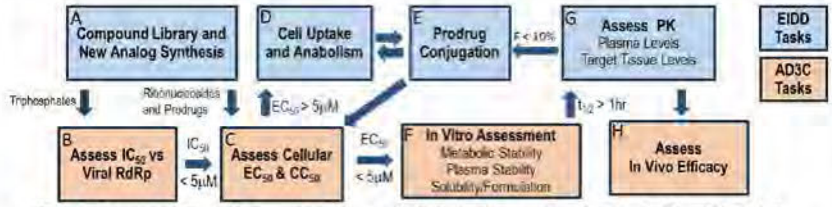


Fig. 4. A comprehensive approach to antiviral ribonucleoside lead identification.

Scale-up Synthesis to Support in Vivo Studies. Experienced synthetic chemists are capable of synthesizing quantities of material ranging from milligram to > 5 gram quantities. SR and EIDD are equipped with synthetic and analytical instruments which will be readily available to assist with the total synthesis required of any compound that is moving forward into additional studies.

B. Bioanalytical Group: This group will be responsible for carrying out *in vitro* ADME. Experimental ADME studies required for medicinal chemistry lead optimization typically include the determination of the solubility and metabolic stability of a compound during the SAR process. When needed, as for VEEV project, blood-brain barrier permeability, plasma protein binding and cytochrome P450 inhibition assessments will also be carried out at different stages of lead optimization. These properties are critical to ensure that a potential new chemical entity is drug-like and possesses the properties required for advancement into pre-clinical development. Following are some of the studies that will be carried out at SR.

Aqueous Solubility:[5] Kinetic solubility of test compounds in PBS at pH 7.4 will be evaluated.
Microsomal Metabolic Stability:[6] It will be assessed in mouse and human liver microsomes.
Log D:[7] A measurement of a compound's lipophilicity which can influence tissue distribution, absorption and binding properties of a compound will be evaluated.
Plasma protein binding:[8] The binding of compounds to plasma proteins influence drug distribution and other PK parameters and fraction unbound as well as recovery levels will be reported.
Metabolite Identification:[9] Early-stage drug metabolite(s) identification is advantageous where radio-labelled drugs are not available. This information suggests specific sites of transformation based on literature precedent and chemical logic and can lead to the synthesis of compounds to block metabolism at the proposed sites.
Permeability:[10] This will be done in MDCK or Caco-2 cells. Test agents showing poor transport across cells will likely show poor oral absorption *in vivo*. Apparent permeability in both directions (A to B and B to A) and efflux ratio will be reported.
Cytochrome P450 (CYP) Inhibition:[11] An *in vitro* screening assay to measure the potential of the test compound to inhibit CYP enzyme (isozymes include CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) activity and to check potentially drug-drug interaction liability.

***In vivo* PK Profiling.** The MCLDC will coordinate all *in vivo* PK studies of compounds using well-established protocols at Pharmaron Inc. SR has worked with Pharmaron on multiple projects to determine PK and they are an experienced subcontractor who has generated consistent, timely, and reproducible results. Prior to administration to animals, each test compound will be prepared in a vehicle having the capacity for dissolving it while lacking appreciable toxicity to the animals being dosed. In selecting vehicles for evaluation, the physicochemical properties of the compound and the intended route of administration will be considered. The PK profile of a compound will be assessed in the same mice strain as that will be used for a particular virus. Properties, such as plasma half-life ($t_{1/2}$), peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), systemically available fraction of a compound (bioavailability %F), clearance (CL), volume of distribute (V_d) and area under the curve (AUC_{inf} and AUC_{last}) will be determined. For VEEV compounds, *in vivo* brain drug concentration will be also be determined. In absence of acceptable exposure, other administration routes such as IV, SC and IP will be performed. Samples from these PK studies will be analyzed by HPLC-MS/MS to determine the concentrations of intact test compound and metabolites of the compound, as appropriate. Relevant PK parameters and oral bioavailability will be calculated for each test compound by WinNonlin®.

C. Structural Biology and Computational Group. This group will be responsible for carrying out protein purification, structural determination and compound design using the technique of x-ray crystallography, and computational approaches as per needs of the projects. At this time, targets of inhibitors in two projects, Alphaviruses and Flaviviruses, are identified and being studied to design more potent analogs of the lead compounds. The group is capable of large-scale protein expression and purification, high throughput crystallization and structural determination of protein-compound structure by x-ray crystallography. Necessary proteins will be purified to develop assays, e.g., nsP3 and nsP2 for CHIKV and VEEV, respectively. Computational techniques will be used as appropriate, and QSAR, CoMFA, and pharmacophore modeling, for example, are all useful tools that can suggest new chemical modifications, including new scaffolds. Where appropriate x-ray or homology data is available as in the case of capsid in DENV and nsP3 protein in CHIKV, structure-based design will be used to assist with the rational design of target compounds. Computational tools, such as (b) (3) (A), (b) (4)™, are also available to generate compounds for synthesis that broadly explore diversity space.

D. Compound and Data Management Group. Tracking of compound submissions to the Assay Core as well as provision of samples to the Center's participants for testing in various assays will be of utmost importance. All synthesized compounds will be fully characterized using standard spectroscopic and chromatographic tools (HPLC, LC/MS and NMR) and the associated antiviral and PK data will be available through an electronic notebook system integrated with a compound management system (b) (3) (A), (b) (4). Each compound will be assigned a unique identifier (SRI number). This number will be used throughout the Center to track compounds and the associated biological and PK data. The MCLDC will also be responsible to perform a freedom-to-operate analysis and coordinate the filing of any patent application. Compounds coming from EIDD will also be distributed to different research project sites via the SR compound management group. SR will use a secured (b) (3) (A), (b) (4) online system for data sharing with Project sites and Administrative Core.

Potential Problems and Alternate Strategies. We have outlined a strategy that should lead to soluble and metabolically potent analogs, and therefore achieve the deliverables described. However, it is common that optimization for one parameter may negatively impact another parameter and various modifications will need to be evaluated to achieve the right balance between the desired potency and the preferred drug-like properties.

We are cognizant of this and will optimize efficiently and effectively, using our reiterative medicinal chemistry and PK knowledge to put forth the best inhibitor(s) for further development. Of course, it is possible that compounds or chemical series cannot be optimized for acceptable drug-like properties with the preferred biological activity. We will consistently make go/no go decisions as we execute lead optimization and will discuss any issues or concerns we may have with the Research Project Site Leaders, Cores and Scientific Advisory Committee.

Key Personnel:

Ashish K. Pathak, Ph.D. will serve as the MCLDC Core Lead who is an Advanced Research Scientist in the Chemistry Department at SR. Dr. Pathak also holds an Adjunct Professor appointment in the Chemistry Department at UAB. He has over 27 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective drug design and vaccine adjuvants with over 65 research publications, patents and patent applications. His experience includes all aspects of a medicinal chemistry program, which extends across the spectrum of early lead discovery to lead optimization through several externally- and in-house funded drug discovery programs. He serves as the current Project Leader of MCLDC of the AD3C. In collaboration with the Project and Core Leaders and Co-Leaders in the Center, he will be responsible for directing all medicinal chemistry, PK, cheminformatics and molecular modeling efforts for the drug discovery effort. He will also be responsible for generating reports and manuscripts, patent application filing and overall project management.

(b) (3) (A), (b) (6)***, Ph.D.***, (b) (3) (A), (b) (6) of the Drug Discovery Division at SR will serve as Co-Core Lead for MCLDC. He has more than (b) years of drug discovery and management experience, serving in a range of capacities, from bench medicinal chemist to leading drug discovery programs at major pharmaceutical (b) (3) (A), (b) (6) as well as smaller biotech and venture-backed organizations. As a medicinal chemist, he is not restricted to a single therapeutic area and relies on collaborations with fellow investigators for developing novel targets and approaches and for using various methodologies such as HTS-driven or virtual screening to identify hits or leads that can be optimized and advanced into animal models. He is experienced with drug discovery and development, and has had a focus on discovering, developing, and advancing new compounds to the clinic in an efficient manner, ensuring that all of the needed data for regulatory filings are properly gathered. He will advise routinely to advance drug discovery efforts in this program.

(b) (3) (A), (b) (6)***, Ph.D.***, Director of SR's Chemistry Department, will also serve as Co-Core Lead for MCLDC. (b) is extensively trained as a medicinal chemist, and an accomplished leader with nearly (b) years of drug discovery and management experience covering at least ten different therapeutic areas. (b) has been responsible for multi-disciplinary project teams at sites worldwide from which clinical candidates have been identified in areas such as Alzheimer's disease, anxiety and inflammation. (b) has been an active member of NIH study sections for several years, has served as a faculty sponsor and mentor to graduate and undergraduate students and currently holds an (b) (3) (A), (b) (6) (b) will advise and actively participate to advance drug discovery efforts in this program.

Additional Personnel:

(b) (3) (A), (b) (6), **Ph.D.**, (b) (3) of Drug Innovation Ventures at (b) (3), (b) (3) (A), (b) (6), will bring to the program (b) years of experience in discovery and development of pharmaceutical agents in the biotechnology and pharmaceutical industry, and extensive experience in nucleoside antivirals. (b) (3) (A), (b) will work closely with the SR team, leading the EIDD group. Other senior staff of the SR Chemistry Department who will participate in MCLDC are: (b) (3) (A), (b) (6) Ph.D., Research Scientist, with more than (b) years of research and synthetic experience in the areas of anticancer, antiviral, nucleoside chemistry and small-molecule drug discovery; (b) (3) (A), (b) (6) Ph.D., Senior Scientist, with more than (b) years of research, bioanalytical, and DMPK experience at SR and the biotech industry performing studies to support drug discovery and clinical development programs; (b) (3) (A), (b) (6) Ph.D. Senior Scientist, with over (b) years of experience in protein sciences including protein identification, expression, purification and functional characterization, and protein structure determination through X-ray crystallography; and (b) (3) (A), (b) (6)**,** Ph.D. Computational Chemist, who will provide molecular modeling/computational chemistry support including conducting structural modeling experiments, protein structure prediction, *in vitro* ADME predictions and calculations.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

The chemistry team will coordinate all *in vivo* pharmacokinetic (PK) studies of selected compounds using standard, well-established protocols at [redacted] (b) (3) (A), (b) (6) an experienced and reputable subcontractor. The Chemistry Department at Southern Research has used these services consistently for several years and generated timely, reliable and reproducible results [redacted] (b) (3) (A), (b) (6)), (b) (6)

1. Description of Procedures.

Laboratory animals will be used to provide a PK assessment of the test compound which can then be used to help understand the PK properties in humans. All studies will be done under non-GLP conditions. Mice are commonly used to perform pre-clinical PK studies. Various mice strains will be chosen based on the corresponding mouse strain (C57BL/6 for CHIK, VEE and SARS viruses, BALB/c for Influenza or A129 for DENV and ZIKV) used for the efficacy studies for each virus. Each PK study will include 5 groups and each group will have 6 mice (male and female equally represented, weight ~30 g each at day 0, 6-8 weeks age) as described in Table A. Dose formulation and dosing methods are described in Table B. Blood samples will be collected as described in Table C. Concentrations of compounds in plasma will be analyzed using a LC-MS/MS method.

Table A. Dosing Information

The study groups are shown in the following table

Group	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Conc. (mg/mL)	Administration Route	No. of Animals
1	Test Compound	1	5	0.2	IV	6
2		5	5	1	IP	6
3		5	5	1	SC	6
4		5	10	0.5	PO_A	6
5		10	10	1	PO_B	6

Animal feeding control: All animals for IV, IP, SC and PO administration will be fed prior to dosing.

Table B. Dose Formulation

Formulation Frequency:	Freshly prepared on of the day of dosing
Vehicle Composition:	20%DMSO 45%PEG400 in 30% HP-β-CD for IV, IP and SC 20%DMSO 45%PEG400 in Water for PO_A and PO_B
Storage Condition:	Dose formulation for dosing: Room temperature
Dosing Site	For IV dosing, the animals will be administered intravenously via tail vein. For IP dosing, the dose formulation will be administered through peritoneal cavity. For SC dosing, the animals will be administered subcutaneously. For PO dosing, the animals will be administered via oral gavage.

Table C. Blood Sample Collection and Processing

Collection Site:	Dorsal metatarsal vein
Volume Collected:	~ 0.03 mL per time point
Anticoagulant:	Heparin Na anti-coagulant tube
Samples Processing and Storage:	1) Approximately 0.03 mL blood will be collected at each time point. Blood of each sample will be transferred into plastic micro centrifuge tubes containing anticoagulant of Heparin-Na. 2) Blood samples will be centrifuged at 4,000 g for 5 minutes at 4°C to obtain plasma. 3) The samples will be stored in a freezer at -75±15°C prior to analysis.

2. Justifications

The aim of the study is to identify the PK profile of the test articles in mice after PO, IV or other routes of administrations. Although *in vitro* methods have been developed to screen PK characteristics of the compounds, those methods are less predictive than a systematic animal study. The laboratory animals used in these studies provide the best means of assessing the PK of the test articles intended for human use. The information to be obtained from these studies will serve as important references for drug development in humans.

3. Minimization of Pain and Distress

Maintenance and care of animals will be in accordance with the AAALAC standards and will meet or exceed requirements established in the *Guide for the Care and Use of Laboratory Animals* (2011). All efforts needed to minimize discomfort to the animals will be made. The animal technicians involved in any way in the proposed research have had instruction and have demonstrated their competence in the care, use and handling of laboratory animals. Mice will be euthanized using IACUC approved methods which are consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.

1. Clark, D.E. In silico prediction of blood-brain barrier permeation. *Drug Disco. Today* 2003, *8(20)*, 927-933.
2. Deli, A.A.; Abraham, A.S.; Kataoka, Y.; Niwa, M. Permeability studies on in vitro blood-brain barrier models. *Cell. Mol. Neurobiol.* 2005, *25(1)*, 59-125.
3. van de Waterbeemd, H., Smith, D. A., Beaumont, K., & Walker, D. K.. Property-based design: Optimization of drug absorption and pharmacokinetics. *J. Med. Chem.* **2001**, *44*, 1313–1333.
4. Lavie, A.; Konrad, M., Structural requirements for efficient phosphorylation of nucleotide analogs by human thymidylate kinase. *Mini-Reviews in Med. Chem.* **2004,** *4(4)*, 351-359.
5. Bevan, C.D.; Lloyd, R.S. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. *Anal. Chem.* **2000**, *72(8)*, 1781-7.
6. Fonsi, M.; Orsale, M.V.; Monteagudo, E. High-throughput microsomal stability assay for screening new chemical entities in drug discovery. *J. Biomol. Screen.* **2008**, *13(9)*, 862-9.
7. Alelyunas, Y.W.; Pelosi-Kilby, L.; Turcotte, P.; Kary, M.B.; Spreen, R.C. A high throughput dried DMSO LogD lipophilicity measurement based on 96-well shake-flask and atmospheric pressure photoionization mass spectrometry detection. *J. Chromatogr. A.* **2010**, *1217(12)*:1950-5.
8. Kariv, I.; Cao, H.; Oldenburg, K.R. Development of a high throughput equilibrium dialysis method. *J. Pharm. Sci.* **2001**, *90(5)*, 580-87.
9. Zhu, M.; Ma, L.; Zhang, D.; Ray, K.; Zhao, W.; Humphreys, W.G.; Skiles, G.; Sanders, M.; Zhang, H. Detection and characterization of metabolites in biological matrices using mass defect filtering of liquid chromatography/high resolution mass spectrometry data. *Drug Metab. Dispos.* **2006**, *34(10)*, 1722-33.
10. Wang, Z.; Hop, C.E.; Leung, K.H.; Pang, J. Determination of in vitro permeability of drug candidates through a caco-2 cell monolayer by liquid chromatography/tandem mass spectrometry. *J. Mass Spectrom.* **2000**, *35(1)*, 71-6.
11. Bjornsson, T.D. *et. al.* The conduct of *in vitro* and *in vivo* drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab. Dispos.* **2003,** *31(7)*, 815-32.

RESEARCH SITES MEDICINAL CHEMISTRY AND LEAD DEVELOPENT CORE (CORE C)

The Core has two Research Sites:

Primary Site: Southern Research, Birmingham, AL
Additional Site: Emory University, Atlanta, GA

Both sites have a separate component in ASSIST, allowing them to upload separate documents for:

- Facilities & Other Resources
- Equipment
- Sr/Key Personnel
- Research Budget and Budget Justification
- Vertebrate Animals
- Resource Sharing

Other documents have been jointly developed and are uploaded under the Primary Site component; where omission of a document would create an error, a placeholder document has been uploaded that refers to the Primary Site. It concerns the following:

- Bibliography & References Cited
- Specific Aims
- Research Strategy
- Authentication of Key Biological and/or Chemical Resources

Finally, the same Project Summary has been uploaded for both Research Sites.

The MCLDC will generate no cell lines or model organisms and, therefore, a plan for sharing these types of materials is not applicable to the current application. The primary output of the MCLDC will be synthesized compounds, specifically new chemical entities as potential new drug candidates and the corresponding efficacy, toxicity, PK/ADME, and other biological data of selected compounds. All novel chemical matter will be appropriately covered in patent applications and all relevant data will be subsequently published in scientific journals. Once published, and as available, we will make research samples of synthetic compounds available to the scientific community for use as chemical probes and for other biological studies.

Data documentation. All research data collected during the grant period will be documented in various forms, including in laboratory notebooks and electronically. Compound structures and screening data will be archived electronically using (b) (3) (A), (b) (4)

Public disclosure. The sharing of final research data will occur by oral disclosure and written scientific publications after appropriate patent protection is in place. These methods are effective means of sharing data which also provide an opportunity for peer-review.

- Oral disclosure. The scientists engaged in the research described in this proposal actively participate in scientific meetings, where current data are freely shared.
- Publication. We expect that final research data will be shared in peer-reviewed scientific journals. Such journals are widely read by scientists engaged in antiviral and biodefense research as well as in other fields. Therefore, we anticipate that publication in journals will permit broad sharing of research data.

Protection of Intellectual Property Rights. Consistent with the policies at the participating institutions of this proposal and NIH policy on intellectual property rights, the laboratories of the key personnel will make all materials produced under this Project available for use at other academic or not-for-profit institutions at no cost, except for standard maintenance and transportation expenses. Southern Research, UAB, and other collaborating institutions will reserve the right to use these reagents for education, research, or other not-for-profit purposes. These institutions may establish non-exclusive commercial licenses granting their rights to use the materials at specific for-profit entities; in these cases, the institutions will maintain the right to grant non-exclusive licenses for the use of these materials by academic or not-for-profit institutions. Requests for materials from for-profit corporations to use them commercially may be negotiated by the Intellectual Property Group of the participating institutions in collaboration with the other institutions as necessary.

1. Chemicals.

Commercially available chemicals and reagents will be purchased at the highest quality possible from reputable vendors. The reagents will be stored according to manufactures instructions. Synthesized compounds will be prepared in >95% purity, and the structure and purity will be examined by NMR, mass spectroscopy and HPLC. The informatics and compound registration database (b) (3) (A), (b) (4) at SR will be used for data capture, analysis and storage of all newly synthesized compounds with a unique compound identifier (SRI number).

2. Mouse Strains.

Animals will be purchased by (b) (3) (A), (b) (4) from fully-accredited vendors approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and will be stored per those instructions. The following three mice strains will be used in this project and will be chosen based on the types of viruses:

C57BL/6 Mice – CHIKV, VEEV and SARS virus
BALB/c mice – Influenza
A129 Strain Mice – DENV and ZIKV

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0664699330000

Legal Name*: Emory University
Department: Emory Institute of Drug Dev
Division: (b) (3) (A), (b) (6)
Street1*: 1599 Clifton Road NE, (b) (3) (A), (b) (6)
Street2:
City*: Atlanta
County: DeKalb
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 30322-4250

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Ms.	(b) (3) (A), (b) (6)		(b) (3) (A), (b) (6)	

Position/Title: Director, Pre-award Grants Adm
Street1*: 1599 Clifton Road NE, (b) (3) (A), (b) (6)
Street2:
City*: Atlanta
County: DeKalb
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 30322-4250
Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6) Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Medicinal Chemistry and Lead Development Core - EIDD

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Emory University, (b) (3) (A), (b) (6) Research Center
Duns Number: 0664699330000
Street1*: 954 Gatewood Rd NE
Street2:
City*: Atlanta
County: DeKalb
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 30329-4208
Project/Performance Site Congressional District*: GA-005

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	MCLDC-Core_C-Abstract.pdf
8. Project Narrative*	
9. Bibliography & References Cited	
10.Facilities & Other Resources	Facilities.pdf
11.Equipment	Equipment.pdf

The Medicinal Chemistry and Lead Development Core (MCLDC) is a key component of the Antiviral Drug Discovery and Development Center (AD3C) that will contribute to the goals of the overall Center of Excellence for Translational Research (CETR) program to develop new replication inhibitors and other broad-based therapeutics for the treatment of emerging pathogens through its interaction with each of the individual projects and cores. The MCLDC will provide a) synthetic chemistry, b) structure-activity relationship (SAR) data and analysis, c) lead optimization chemistry, d) bioanalytical assays, e) structural biology and computational support, and d) compound storage and data management. In this role, the MCLDC, in conjunction with the Assay Core, will be the central focus of the translational research component of the program. As such, the MCLDC will work closely with the Assay Core and each of the Research Project teams. As new chemical entities are designed and synthesized during the lead generation and optimization processes, the Assay Core will test these analogs in SAR-driving assays. Testing of compounds for drug-like properties such as microsomal stability and solubility; and *in vitro* Absorption, Distribution, Metabolism and Excretion (ADME) will be an integral part of SAR studies from the early developmental phase. Appropriate lead compounds will be provided to the various Research Project teams for advanced studies including efficacy, mechanism of action, and other experiments. The resulting data on lead compounds from the Assay Core and Research Projects will then be analyzed by the MCLDC to drive the iterative lead optimization and *in vivo* PK studies to completion, resulting in optimized leads with drug-like properties for animal studies. The lead candidates will have met the go/no go decision criteria set for activity, potency and drug-like properties. The goal for the end result of this process will be identification of novel optimized lead molecules that are appropriate for IND applications. The MCLDC will incorporate key personnel of other Cores and Research Project teams into the prioritization and decision–making procedures.

(b) (3) (A), (b) (4), (b) (6)

[redacted]

(b) (3) (A), (b) (4), (b) (6)

[redacted]

(b) (3) (A), (b) (4)

[redacted]

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name (b) (3) (A), (b) (6) Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Director, Professor of Pharmacology
Organization Name*: Emory University
Department: (b) (3) (A), (b) (6)
Division:
Street1*: 954 Gatewood Rd NE
Street2:
City*: Atlanta
County:
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 303294208

Phone Number*: (b) (3) (A), (b) (6) Fax Number:

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Subcontract PI

Degree Type: PHD Degree Year: 1977

Attach Biographical Sketch*: File Name: (b) (3) (A), (b) (6)_Biosketch.pdf

Attach Current & Pending Support: File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted] (b) (3) (A), (b) (6)				Subaward PI	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			3,792.00	1,035.00	4,827.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **4,827.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	[redacted] (b) (6)			99,253.00	27,096.00	126,349.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Scientist	[redacted]			9,085.00	2,480.00	11,565.00
1	Research Specialist	[redacted]			28,232.00	7,707.00	35,939.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**173,853.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**178,680.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0664699330000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Emory University
Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0664699330000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Emory University
Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	53,020.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care (per diems)	300.00
9. Maintenance Contracts	18,000.00
Total Other Direct Costs	**71,320.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	78.5	250,000.00	196,250.00
		Total Indirect Costs	**196,250.00**

Cognizant Federal Agency DHHS, Steven Zuraf (301)492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**446,250.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	446,250.00

L. Budget Justification* File Name:
Emory_Core_Budget_Justification_2018-0322_a.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted]		(b) (3) (A), (b) (6)		Subaward PI	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			3,906.00	1,066.00	4,972.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **4,972.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			102,232.00	27,909.00	130,141.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Scientist	[redacted]			9,358.00	2,555.00	11,913.00
1	Research Specialist	[redacted]			29,079.00	7,939.00	37,018.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**179,072.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**184,044.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	47,656.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care (per diems)	300.00
9. Maintenance Contracts	18,000.00
Total Other Direct Costs	**65,956.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	78.5	250,000.00	196,250.00
		Total Indirect Costs	**196,250.00**

Cognizant Federal Agency DHHS, Steven Zuraf (301)492-4855

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**446,250.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	446,250.00

L. Budget Justification* File Name:

Emory_Core_Budget_Justification_2018-0322_a.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted] (b) (3) (A), (b) (6)				Subaward PI	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			4,023.00	1,098.00	5,121.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **5,121.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			105,298.00	28,746.00	134,044.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Scientist	[redacted]			9,639.00	2,631.00	12,270.00
1	Research Specialist	[redacted]			29,952.00	8,177.00	38,129.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**184,443.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**189,564.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	42,136.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care (per diems)	300.00
9. Maintenance Contracts	18,000.00
Total Other Direct Costs	**60,436.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	78.5	250,000.00	196,250.00
		Total Indirect Costs	**196,250.00**

Cognizant Federal Agency DHHS, Steven Zuraf (301)492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**446,250.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	446,250.00

L. Budget Justification* File Name:
Emory_Core_Budget_Justification_2018-0322_a.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted] (b) (3) (A), (b) (6)					Subaward PI	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			4,144.00	1,131.00	5,275.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **5,275.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			108,456.00	29,608.00	138,064.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Scientist	[redacted]			9,928.00	2,710.00	12,638.00
1	Research Specialist	[redacted]			30,850.00	8,422.00	39,272.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**189,974.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**195,249.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	36,451.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care (per diems)	300.00
9. Maintenance Contracts	18,000.00
Total Other Direct Costs	**54,751.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	78.5	250,000.00	196,250.00
		Total Indirect Costs	**196,250.00**

Cognizant Federal Agency DHHS, Steven Zuraf (301)492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**446,250.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	446,250.00

L. Budget Justification* File Name:
Emory_Core_Budget_Justification_2018-0322_a.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted] (b) (3) (A), (b) (6)				Subaward PI	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			4,268.00	1,165.00	5,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **5,433.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	[redacted] (b) (6)			111,710.00	30,497.00	142,207.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Scientist	[redacted]			10,226.00	2,792.00	13,018.00
1	Research Specialist	[redacted]			31,776.00	8,675.00	40,451.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**195,676.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**201,109.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	30,591.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Care (per diems)	300.00
9. Maintenance Contracts	18,000.00
Total Other Direct Costs	**48,891.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	78.5	250,000.00	196,250.00
		Total Indirect Costs	**196,250.00**

Cognizant Federal Agency DHHS, Steven Zuraf (301)492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**446,250.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	446,250.00

L. Budget Justification* File Name:
Emory_Core_Budget_Justification_2018-0322_a.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

B. BUDGET JUSTIFICATION

(b) (3) (A), (b) (6) **Ph.D.** (sub-Principal Investigator), (b) (6) cal. months/year. (b) (3) (A), (b) (6) has (b) years of experience in the discovery and development of pharmaceutical agents in the biotechnology and large pharmaceutical company sectors. He has been a member of the founding management teams of two highly successful biotechnology companies, (b) (3) (A), (b) (6) is currently running advanced development contracts for the U.S. government worth a total of $124 million. Prior to his management experience in biotechnology, (b) (3) (A), (b) held positions of increasing responsibility in the pharmaceutical industry beginning in 1982 including (b) (3) (A), (b) (6) He has published over (b) scientific papers and is listed as an inventor on more than (b) patents, (b) of which have led to approved drugs or combinations of drugs for the treatment of HIV and hepatitis B. He has led international drug development teams, which generated data for (b) (3) (A), applications and (b) (3) (A), (b) (6). He currently serves on the (b) (3) (A), (b) (6)). He earned a B.S. in chemistry, an M.S. in physical organic chemistry and a Ph.D. in synthetic organic chemistry from (b) (3) (A),

(b) (3) (A), (b) will serve as the Principal Investigator for the Emory subcontract on this proposed program. He will bring to bear his extensive experience for the development of a safe and effective ribonucleoside based therapy for the identification of new antiviral ribonucleosides and ribonucleotides. He will be responsible for overall strategic and scientific oversight as well as coordination of the multidisciplinary drug discovery team that has been assembled for the project.

(b) (3) (A), (b) (6)**, PhD,** (Researcher; Nucleoside Chemistry Team Leader) (b) (6) calendar months/year, is an experienced synthetic and medicinal chemist with (b) years of experience as the chemistry team leader for the nucleoside analog program. His training included extensive experience with the synthesis of nucleoside analogs as well as other medicinally active agents to target HCV and drug resistant forms of HIV. He will serve as the chemistry team leader for the duration of this program and he will oversee the day-to-day operation of the chemistry team and coordinate efforts between disciplines.

(b) (3) (A), (b) (6)**, Ph.D.** (Postdoctoral Fellow), (b) (6) cal. Months/year is an experienced synthetic chemist who has over a year of experience synthesizing nucleoside and nucleotide based compounds at small and large scale. He has developed unique techniques that are critical for the challenging task of synthesizing highly polar nucleoside compounds and their nucleotide counterparts.

(b) (3) (A), (b) (6)**, Ph.D.** (Postdoctoral Fellow), (b) (6) cal. months, is an analytical chemist with (b) years of experience in the pharmaceutical industry and (b) (3) years of experience at the Emory Institute for Drug Development. As the lead specialist in the bioanalytical chemistry team of the EIDD, she will be responsible for the bioanalyses of cellular and tissue samples generated at EIDD and the labs of our collaborators in this proposed program.

TBD. (Postdoctoral Fellow), 6 cal. months in years 1 – 3, 4.2 cal. months in year 4, 1.8 cal. months in year 5. A portion of a post doctoral chemist's effort will be required to meet the milestones set out in this proposal. The effort required will diminish in the later years as the medicinal chemistry effort comes to fruition.

(b) (3) (A), (b) (6)**,** (Molecular Biologist), (b) (6) calendar months/year, is a senior research specialist at the Emory Institute for Drug Development with over (b) (3) (A), (b) years of research experience in both the pharmaceutical industry and academia. (b) (3) (A), (b) (6) has experience in DNA and RNA isolation, polymerase chain reaction (PCR) assays, transformation of competent E. Coli, DNA sequencing, Northern and Southern Blots, In situ hybridization, growth, harvesting and lysis of bacteria culture, extraction and purification of plasmid DNA, ligation reactions, gel electrophoresis, identification and analysis of recombinants and genotyping. During (b) (3) (A), (b) (6) time at the EIDD, (b) (3) (A), (b) (6) has also held primary responsibility for the dosing and sampling of small animals for pharmacokinetic and toxicokinetic experiments. In this proposed project, (b) (3) (A), (b) (6) will carry out cellular uptake and anabolism experiments, and provide assistance and supervision in the *in vivo* phases of PK and TK experiments.

Salaries are inflated 3% in years 2-5. The current fringe rate for Emory University is 27.3%.

Supplies

Total consumable supplies and reagents in period 1 = $46,625
Total consumable supplies and reagents in period 2 = $41,101
Total consumable supplies and reagents in period 3 = $35,383
Total consumable supplies and reagents in period 4 = $43,098
Total consumable supplies and reagents in period 5 = $37,439

**Throughout the program, a composite of standard reagents, solvents and consumable equipment is budgeted at a rate of ~$1,000 per month per working chemist, $1,400 per month per working bioanalyst, and ~$2,000 per month per working biologist; which, represents the average cost of such items for EIDD chemists over the course of 2014 - 2017.*

Costs for 40 cannulated rats per year ($98 per rat) are also included to support PK and tolerability studies with emerging hit candidates
Costs for 108 mice per year ($8.40 per mouse) are also included to support tissue distribution studies with active compounds that demonstrate good PK in rats

Other Costs
$18,000 / year are included in the budget to cover 15% of the maintenance fees for the bioanalytical instruments, which is anticipate to be the total proportion of usage of the instruments to cover work for this program.
$300 / year is included to cover the anticipated per diem fees for animal studies

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		25,628.00
Section B, Other Personnel		923,018.00
Total Number Other Personnel	25	
Total Salary, Wages and Fringe Benefits (A+B)		948,646.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		301,354.00
1. Materials and Supplies	209,854.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	1,500.00	
9. Other 2	90,000.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,250,000.00
Section H, Indirect Costs		981,250.00
Section I, Total Direct and Indirect Costs (G + H)		2,231,250.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		2,231,250.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Refer_to_SR_Aims.pdf
3. Research Strategy*	Refer_to_SR.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_U19_Whitley.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Core_C.pdf
9. Letters of Support	
10. Resource Sharing Plan(s)	Resource_Sharing_Plan.pdf
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

For this document, please refer to the one submitted for the Primary Site for this Core, Southern Research.

For this document, please refer to the one submitted for the Primary Site for this Core, Southern Research.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

Vertebrate Animals

Animal Rodent Models for Pharmacokinetics and Tissue Distribution Studies

1) Detailed description of proposed use of animals

Pharmacokinetics (PK) Model in Rats
Species: *Rattus norvegicus*
Strain: Sprague Dawley (SD)
Ages: 7-8 weeks old (~250g)
Sex: Male
Numbers: 40 /year
The purpose of these animal experiments is to determine the PK parameters of the test compounds. PK studies will be used to test the leads for oral bioavailability and the rates of clearance from the body. These studies will be done in pre-cannulated rats (the jugular vein cannula will be inserted by the vendor) with timed blood collections at 0.08, 0.25, 0.5, 1, 2, 3, 6 and 24 hours after intravenous (IV) dosing and 0.5, 1, 2, 3, 4, 6, 8 & 24 hrs after oral (PO) dosing. Blood will be sampled through the cannulas. The work will be done in the animal facility of the Neuroscience building of the (b) (3) (A), (b) (6)

Animal numbers: Rats will be used for PK experiments to test new leads. The PK/ studies will be done for two compounds per year, using three PO dose groups and one IV group each of 4 rats plus one control (vehicle) rat ordered for each group, for a total of 5 X 4 [doses] X 2 [compounds] = 40 rats/year.

Pharmacokinetics/Pharmacodynamics (PK/PD) Model in Mice
Species: *Mus musculus.*
Strain: CD-1
Ages: 6-8 weeks old (~25 g ordered)
Sex: Male and female
Numbers: 108 female and 27 male mice/year
The purpose of these animal experiments is to determine the PK/PD parameters of the test compounds. PK/PD studies will be used to test selected leads with acceptable PK parameters for mouse PK and tissue distribution. These studies will be done in mice with timed blood and organ collections at 0.5, 1, 2, 3, 4, 6, 8 & 24 hrs after PO dosing and at 0.08, 0.25, 0.5, 1, 2, 3, 6 and 24 hours after IP dosing. Blood will be sampled by retroorbital bleeding under anesthesia; mice will then be immediately euthanatized and internal organs will be collected: brain, heart, lung, liver, spleen and kidney. The work will be done in the animal facility of the Neuroscience building of the (b) (3) (A), (b) (6).

Animal numbers: Mice will be used for PK/PD experiments to test selected leads. The PK/PD studies will be done for one compound per year, using three PO dose groups and one IP group of 3 mice/timepoint/compound for a total of 3 [mouse-samples] X 4 [doses] X 9 [timepoints+1 control] = 108 female mice/year and using one PO dose group of 3 mice/timepoint/compound for a total of 3 [mouse-samples] X 1 [doses] X 9 [timepoints+1 control] = 27 male mice/year

2) Justification of animal use
Mice and Rats are the preferred species for preclinical PK/PD studies, due to their moderate cost and usefulness for extrapolating results from rodent animal models to humans. Mice and rats from reputable supplier (b) (3) (A), (b) (6) will be used. For the initial PK studies in cannulated rats, 4 animals/group has proven to be the absolute minimum effective number of animals for this type of experiment, since it allows for calculations of means and standard errors and the traditional set of PK parameters (Cmax, Tmax, AUC, drug half-life, bioavailability, etc.).
For the PK/PD studies in mice, 3 animals/group has proven to be the absolute minimum effective number of animals for this type of experiment, since it allows for calculations of means and standard

errors and the PK/PD parameters. One PO group is planned for male mice to analyze if there are significant differences with female mice. Additional experiments will be conducted if significant differences between female and male PK/PD data will be observed.

3) Veterinary care of animals

Animals for the PK and PK/PD studies will receive routine care from the full-time veterinary and husbandry staff of the AAALAC accredited Emory Department of Animal Resources (DAR) at (b) (3) (A), (b) (6) [redacted]. The animals will be housed in microisolator cages, in groups of three-to-five per cage for mice and rats will be housed single rat per cage; cages are changed under laminar flow hoods. All animal handling personnel of the EIDD have been adequately trained, possess multiple-year experience and have been certified in animal handling and regulations by the Emory DAR.

4) Procedures to limit discomfort, distress, pain and injury

Animals will be monitored continuously during the day as they are dosed and sampled. If any signs of toxicity appear due to the drugs, or, if problems develop with sampling, the animals will be immediately euthanatized with CO_2 according to standard procedures.

5) Method of euthanasia

All animals will be euthanized using an overdose of CO2, which is an American Veterinary Medical Association (AVMA) accepted form of euthanasia. All animals will be checked to ensure that the heart has completely stopped beating and that there is no sign of respiration or corneal reflex.

RESEARCH SITES MEDICINAL CHEMISTRY AND LEAD DEVELOPENT CORE (CORE C)

The Core has two Research Sites:

Primary Site: Southern Research, Birmingham, AL
Additional Site: Emory University, Atlanta, GA

Both sites have a separate component in ASSIST, allowing them to upload separate documents for:

- Facilities & Other Resources
- Equipment
- Sr/Key Personnel
- Research Budget and Budget Justification
- Vertebrate Animals
- Resource Sharing

Other documents have been jointly developed and are uploaded under the Primary Site component; where omission of a document would create an error, a placeholder document has been uploaded that refers to the Primary Site. It concerns the following:

- Bibliography & References Cited
- Specific Aims
- Research Strategy
- Authentication of Key Biological and/or Chemical Resources

Finally, the same Project Summary has been uploaded for both Research Sites.

Resource Sharing Plan

External data sharing

Timely communication of scientific results from this Core will occur through national/international scientific meetings based upon submitted abstracts, and in the form of peer-reviewed scientific publications. We will share any scientific findings discovered through this research Program at the time of publication of results or after completion of a given project. These will be made available to qualified individuals within the scientific community for research purposes. Further, this Core will make all progress and data available to NIAID through semi-annual, written progress reports and attendance annual National CETR Program Meetings and Reverse Site visits.

Peer-reviewed scientific publications

In order to help advance scientific knowledge and improve human health, any manuscript that has been accepted for publication in a peer-reviewed journal and summarizes scientific results originating from this project will be digitally archived in *PubMed Central* at the time of publication.

Model Organisms

NA within this Core

Genome Wide Association Studies

NA within this Core

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0636907050000

Legal Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department:
Division:
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave South, (b) (3) (A), (b) (6)
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	(b) (3) (A), (b) (6)		(b) (3) (A), (b) (6)	

Position/Title:
Street1*: 1720 2nd Avenue South, (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6) Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 1 - Coronavirus

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Duns Number: 0636907050000
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave South, (b) (3) (A), (b) (6)
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352940111
Project/Performance Site Congressional District*: AL-007

Project/Performance Site Location 1

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Vanderbilt University Medical Center
DUNS Number: 0799178970000
Street1*: 3319 West End Avenue
Street2: (b) (3) (A), (b) (6)
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37203-6856
Project/Performance Site Congressional District*:

Project/Performance Site Location 2

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The University of North Carolina at Chapel Hill
DUNS Number: 6081952770000
Street1*: 2107 McGavran Greenberg Hall
Street2: Campus Box #7435
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435
Project/Performance Site Congressional District*:

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 __ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_1_CoV_Summary_3-20-18.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project_1_CoV_Bibliography_3-23-18.pdf
10.Facilities & Other Resources	Facilities_Corona.pdf
11.Equipment	Equipment_Corona.pdf
12. Other Attachments	Product_Development_Strategy.pdf

PROJECT SUMMARY

Zoonotic coronaviruses (CoVs) such as SARS-CoV and MERS-CoV are pandemic threats. MERS-CoV continues to cause new zoonotic and human transmission and illness with ~35% mortality. Currently, there are no FDA-approved therapies to treat any CoV. New zoonotic CoVs likely will emerge from heterogeneous virus pools in animal reservoirs, thus requiring antiviral strategies aimed at completely conserved and vulnerable targets. CoVs rapidly select for resistance to multiple classes of inhibitors, demonstrating the need for approaches to prevent resistance emergence. Both SARS and MERS infections manifest as severe immunopathologic damage, potentially limiting the therapeutic window for direct-acting antivirals (DAAs). Immunomodulation in the absence of antivirals has been shown to not be beneficial and to even exacerbate SARS and MERS disease. Thus, combinations of DAAs and targeted immunomodulators may be necessary for effective treatment of established infection. ***The overall goal of our program is to develop CoV antiviral strategies that broadly inhibit known and future potential pandemic zoonotic CoVs, prevent emergence of resistance, and extend the therapeutic window by targeting host immunopathologic responses.*** The proposed research will advance preclinical development of the CoV-inhibitory nucleoside analogue EIDD-1931/2801 and other nucleoside analogues in the pipeline and test two small-molecule hits identified as highly active against SARS-CoV for treatment and prevention of epidemic and pre-emergent CoVs. In **Specific Aim 1**, the spectrum of antiviral activity and therapeutic efficacy of compounds will be defined. The antiviral efficacy, metabolism, and cytotoxicity of each compound will be determined in cultures of primary human lung cells targeted by SARS- and MERS-CoV. The prophylactic and therapeutic efficacy of lead compounds will be evaluated in young, aged, and immunosuppressed murine models of SARS and MERS pathogenesis. In **Specific Aim 2**, the mechanism of action of lead compounds and kinetics of drug resistance will be determined. The antiviral effect of compounds on virus replication, fidelity, and induction of innate immunity will be assessed. Resistance mutations in genomes of MERS- and SARS-CoV passaged in the presence of increasing concentrations of drug will be determined by deep sequencing. The impact of resistance on SARS- and MERS-CoV virulence, sensitivity to other drugs, and therapeutic efficacy of lead compounds will be determined. **Specific Aim 3** will focus on the development of combination regimens for the treatment of emerging CoVs. The combined therapeutic efficacy of DAAs against infections with both wild-type and drug-resistant SARS- and MERS-CoV will be defined using cultured cells and mice. The therapeutic effect of treatment combining a DAA with an immunomodulator will be assessed in mouse models of SARS and MERS. These studies will generate mechanistic and efficacy data necessary for IND filing and origination of human clinical trials.

(b) (3) (A), (b) (6), (b) (4)

[redacted]

FACILITIES AND RESOURCES [redacted] (b) (3) (A), (b) (6), (b) (4)

(b) (3) (A), (b) (6), (b) (4)

[redacted]

(b) (3) (A), (b) (4)

[redacted]

(b) (3) (A), (b) (4)

[redacted]

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Introduction

(b) (4), (b) (3) (A)

[redacted]

[redacted] (b) (4), (b) (3) (A)

Both in vitro and in vivo data against infections from the respiratory viruses [redacted] (b) (4), (b) (3) (A) [redacted] (b) (4), (b) (3) (A) strongly support investigating the potential of EIDD-1931 [redacted] (b) (4), [redacted] (b) (4), (b) (3) (A). Given the morbidity and high incidence of mortality associated with disease, identification of a treatment for MERS represents an extremely important medical need as there are currently no FDA-approved drugs available for treatment of coronavirus infections.

EIDD-1931 has been under development as an orally bioavailable antiviral agent for several indications. Therefore, it was essential to show oral bioavailability in a range of animal species. The need for data in different species arises in both a conventional development program in which pivotal safety and efficacy data is gathered in human clinical studies (where rats and dogs have been identified as toxicology species for CHKV, RSV, or IAV programs), and in a program where pivotal efficacy data is acquired under the Animal Efficacy Rule (efficacy data in mice and cynomolgus macaques for the VEEV program). [redacted] (b) (4), (b) (3) (A) [redacted] (b) (4), (b) (3) (A) [redacted] (b) (4), (b) (3) (A) Consequently, EIDD-2801 is the compound that will be taken into advanced development. Within the scope of this proposed project, we will characterize the mechanism of activity of EIDD-2801 against coronaviruses and explore the prophylactic and therapeutic effects in relevant murine models of infection.

Outline of Milestones and Timeline. The proposed 5-year research project is designed to characterize and validate the efficacy (both in vitro and in vivo), resistance profile and mechanism of action of EIDD-1931/2801 as an antiviral agent for the prevention and treatment of coronavirus infections, specifically MERS-CoV. The program will be carried out in parallel with ongoing contracts that cover IND-enabling studies for EIDD-1931/EIDD-2801: 1. DTRA contract HDTRA1-15-C-0075 covers the nonclinical development of EIDD-1931 as a medical countermeasure for VEEV infections through preclinical development under the Animal Efficacy Rule; and 2. NIAID contract HHSN272201500008C covers studies that support the use of EIDD-1931/EIDD-2801 for Chikungunya virus infections. The activities of both contract programs are included, for reference, in the timeline below (**Fig. PD1**). The timeline provides an overview of the project proposed in this grant submission in the context of the overall development program for EIDD-1931/2801.

In this submission, we propose a five-year nonclinical program to characterize and support the use of EIDD-1931/2801 to treat coronavirus infections. The data generated from this project can be used along with data from ongoing work to define a dose and dosing regimen of EIDD-2801 for clinical safety and efficacy studies in coronavirus infections.

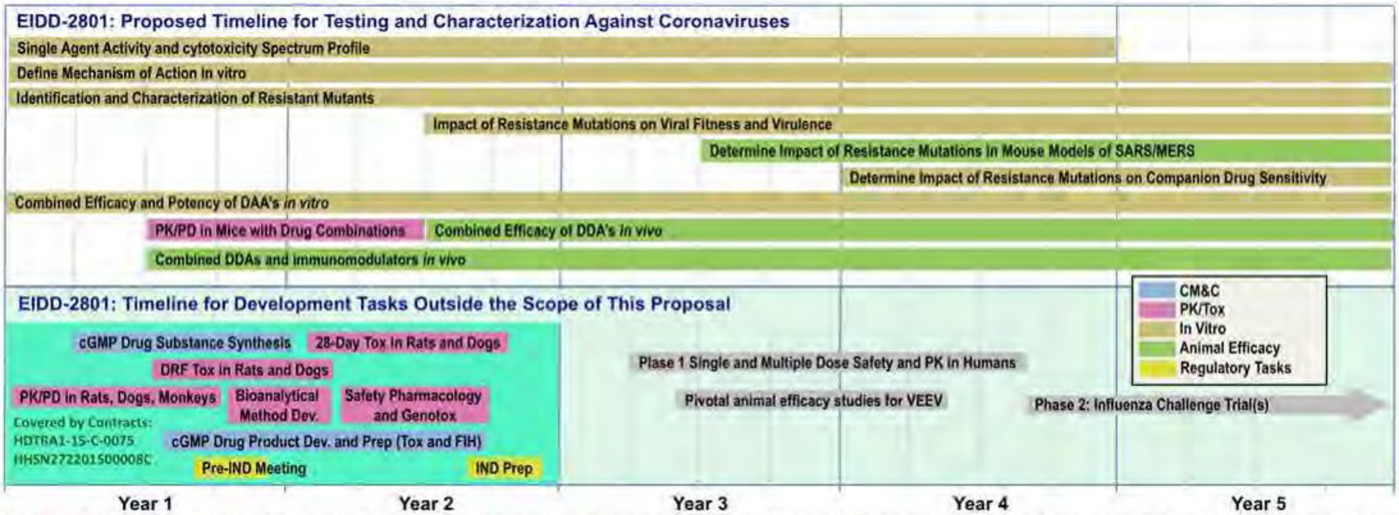


Figure PD1 *– Development plan for the proposed program to support the use of EIDD-2801 as a treatment for emerging CoVs (upper panel), and the parallel development program for EIDD-1931 that is supported by contracts HDTRA1-15-C-0075 and HHSN272201500008C (lower panel).*

The overall development strategy for EIDD-1931/2801 has been designed to enable submission of an IND to the FDA under the Animal Efficacy Rule. When seeking approval under the Animal Rule, clinical safety data must be collected. It is also highly desirable to have human efficacy data from clinical studies in other indications to help establish a recommended dosing paradigm. In this context, two Phase 1 safety studies are planned; one in healthy volunteers (first-in-human (FIH), single (SAD) and multiple (MAD) ascending dose study) and one in subjects experimentally infected with low virulence strains of Influenza virus as outlined by the FDA Guidance for Industry “Influenza: Developing Drugs for Treatment and/or Prophylaxis”. The objectives of Phase 1 clinical development will be to establish a safety and tolerability profile of EIDD-2801 and to begin to define appropriate dose levels and dosing regimens for prophylactic and therapeutic use in a viral respiratory infection. The safety and dosing results from the influenza study can also be used to inform dosing parameters for other infections.

The development plan timeline includes 2 years for IND-enabling, nonclinical work that is being conducted outside of the scope of this proposal. Following IND submission, the conduct of the Phase 1 SAD/MAD FIH trial will require approximately 18 months. Initiation of a Phase 1b/2a influenza challenge trial will follow early characterization of safety and tolerability.

Proposed Research Program: Characterization and Validation of EIDD-1931/2801 as a treatment for Coronavirus Infections

Spectrum of Antiviral Activity and in vivo *Efficacy*

- Determine EIDD-1931 activity spectrum across CoV groups and genera
- Determine EIDD-1931 antiviral efficacy, metabolism, and cytotoxicity in human lung cells.
- Determine *in vivo* efficacy of EIDD-1931 in models of SARS- and MERS pathogenesis.

Resistance and Mechanism of Action Studies

- ➢ *Determine EIDD-1931/2801 mechanism of action*
  - MERS- and SARS-CoV RNA inhibition
  - MERS- and SARS-CoV mutagenesis
  - Induction of antiviral immunity
- ➢ *Determine the impact of EIDD-1931/2801 resistance mutations*
  - MERS- and SARS-CoV replication and fitness in vitro
  - MERS- and SARS-CoV and virulence in mouse models of MERS- and SARS pathogenesis
    Therapeutic efficacy of EIDD-1931/2801 in murine models of MERS- and SARS pathogenesis

Combination regimens for treatment of emerging CoV disease.

- ➢ Direct acting antivirals: EIDD-1931/2801 and remdesivir.
  - Effect on RNA, mutation frequency, and resistance.
  - Efficacy of combined direct-acting antivirals in murine models of SARS and MERS disease.

- Co-administered EIDD-1931/2801 or remdesivir and immunomodulators
  - Therapeutic efficacy in SARS-CoV- and MERS-CoV-infected mice.

IND Enabling Studies and Early Clinical Work: Tasks (➤) and Go/No Go Criteria (●) for each Task

Chemistry, Manufacturing and Controls

- *Develop a scalable synthetic process for drug substance:* Task completed and milestones met (See Point #5 of Product Description)
  - <8 chemical steps
  - >20% overall yield from commercially available starting materials;
  - >95% purity with no single impurity >0.5%
  - Initial release specifications established
  - Initial analytical methods support synthetic development
- *Prepare cGMP drug substance for IND toxicology and early clinical work (~12kg):* (Year 1)
  - Suitable scale-up and manufacturing site is identified
  - Analytical methods established for transfer and validation
  - Material must meet initial release specifications appropriate for early phase development
- *Drug substance stability testing under ambient and accelerated conditions:* (Year 1)
  - Sufficient ambient stability to support IND enabling studies (Detailed specifications TBD)
  - Sufficient accelerated stability to support IND enabling studies (Detailed specifications TBD)
- *Develop formulations suitable for IND-enabling toxicology studies and the subsequent first in human (FIH) clinical trial:* (Years 1-2)
  - No solubility or polymorph issue identified that would severely impact development timeline (Year 1)
  - A formulation for IND-enabling toxicology studies is developed for gavage dosing (Year 1)
  - A drug product suitable for oral dosing in FIH clinical trial is identified (Year 2)

Pharmacokinetics and Pharmacodynamics

(b) (4)

[redacted]

IND-Enabling Toxicology

(b) (4)

[redacted]

- Adverse events or findings related to administration of test compound including any dose-response relationships are acceptable
- Data informs design of subsequent pivotal GLP toxicology studies

➢ *Definitive (GLP) Ames and Chromosomal Aberration Studies:* (Year 2)
  - The compound should have an acceptable profile in genotoxicity assays

➢ *GLP Safety Pharmacology*: (Year 2)
  - No clinically relevant dose limiting adverse CNS effects (Functional Observational Battery)
  - No clinically relevant dose limiting adverse cardiovascular effects (telemetry in beagle dogs and *in vitro* hERG blockade assessment)
  - No clinically relevant dose limiting adverse pulmonary effects (respiratory parameters in rats)

➢ *28-Day GLP Toxicity in Rats and Dogs:* (Year 2)
  - Maximum Tolerated Dose (MTD) and No Observed Effect Level (NOEL) / No Observed Adverse Effect Level (NOAEL) provide an acceptable therapeutic window
  - Dose Limiting Toxicity (DLT), if observed, provides an acceptable therapeutic window
  - Data informs dose selection for first in human clinical studies

Early Clinical Studies (or animal efficacy equivalents per FDA Animal Efficacy Rule)

➢ *Phase I Clinical Safety, Tolerability, and Pharmacokinetics at Single and Multiple Ascending Doses (SAD / MAD clinical study):* (Year 3 – 4)
  - No clinically significant adverse events related to administration of EIDD-2801 seen at projected therapeutic doses that would preclude further clinical development
  - EIDD-2801 is considered generally safe and well-tolerated
  - Pharmacokinetics demonstrate appropriate plasma levels (e.g., Cmax and AUC)

➢ *Phase 2a Virus Challenge Study (Influenza):* (Year 4 – 5)
  - Prevents disease and/or reduces morbidity based on defined endpoints (TBD)

➢ *Conduct pivotal animal efficacy studies to support approval for the prophylaxis of VEEV infection* (Years 3-4)
  - Prevents disease and/or reduces morbidity/mortality in agreed upon animal species based on defined endpoints (TBD)

➢ *Develop concept sheet for CHKV clinical studies* (Year 2)
  - Acceptable treatment regimen and clinical endpoints are defined

Product Development Plan

(b) (4)
[redacted]

The Emory Institute for Drug Development (EIDD) has two current contracts that support IND-enabling studies for EIDD-1931/2801: 1. DTRA contract HDTRA1-15-C-0075 covers early development of EIDD-1931 as a countermeasure for VEEV infections including nonclinical pharmacology and toxicology studies as well as preclinical development under the Animal Efficacy Rule, and 2. NIAID contract HHSN272201500008C covers studies that support the use of EIDD-1931/2801 for the treatment of CHKV and one other RNA virus infection, for example IAV or IBV.

The development plan below describes the current development status of EIDD-1931/2801 and outlines our proposed plans for investigating the use of EIDD-1931/2801 for coronavirus virus infections. We also clearly differentiate the work for which support is being sought from the work that is currently being supported by other contracts.

1. *__Intended use/indication of the proposed product and the biodefense/public health gap the product is intended to fill.__*

The coronavirus project, proposed in this submission, will characterize the nonclinical efficacy and resistance profile of EIDD-1931 and [redacted] (b) (4) EIDD-2801 against coronavirus infections including MERS-CoV (NIAID Category B pathogen), in preparation for safety and efficacy studies in humans. This program will be run in parallel with the ongoing studies described below. An IND for treatment of coronaviruses will be filed following the generation of proof-of-concept data.

The existing development plan for EIDD-2801, as supported by contracts HDTRA1-15-C-0075 and HHSN272201500008C, is to advance the drug candidate, and open an IND, using the data to support development of EIDD-2801 as countermeasure for VEEV infections under the Animal Efficacy Rule. Influenza is being proposed as a disease target due to the ability to obtain human safety and efficacy data in a reasonable and controlled manner by conducting challenge trial(s) with low virulence strains of influenza as outlined in Guidance for Industry "Influenza: Developing Drugs for Treatment and/or Prophylaxis".

2. *__Statement of the value of the project, including lay description of key technology objectives, innovation and advantages compared to competing products, technologies or services__*

Zoonotic coronaviruses (CoVs) such as SARS-CoV and MERS-CoV are pandemic threats, with MERS-CoV continuing to cause new zoonotic and human transmission and illness, resulting in ~35% mortality. With increasing overlap of human and wild animal ecologies, the potential for future severe zoonotic CoV emergence is high. Currently, there are no FDA-approved therapies to treat any human CoV infection. Potent, broadly effective, resistance-averting therapies are urgently needed to protect against emerging, highly pathogenic zoonotic coronaviruses (CoVs), whose genetic diversity can render vaccines and therapeutics ineffective. Among the compounds that are currently under investigation for use against coronaviruses is remdesivir [redacted] (b) (4),

(b) (4)
[redacted]

(b) (4)
[redacted]

(b) (4)

[redacted]

3. *Description of the goal(s) of the project, including one (or more) intermediate products, final product(s) or stage(s) of product development to be completed during the award period. (no specific product profile by licensing indication is needed)*

In this proposed project, we will characterize and support the use of EIDD-1931/2801 for prophylaxis/treatment of coronavirus infections including MERS-CoV. Specifically, we will: 1. Determine the activity spectrum against genetically diverse zoonotic CoV, and measure toxicity in continuous cell lines and HAE cultures; 2. Assess PK/PD, toxicity and efficacy *in vivo* using murine models of CoV disease; 3. Define mechanism of action and resistance; evaluate the impact of resistance on virulence and therapeutic efficacy *in vivo*; 4. Assess the impact of resistance on virus fidelity and sensitivity to other drugs; 5. Determine if combining EIDD-1931/2801 with other direct-acting antivirals leads to increased potency, delayed resistance, and a greater activity spectrum; and 5. Determine if treatment with combinations of EIDD-1931/2801 with other direct-acting antivirals and/or immunomodulators extends the therapeutic window for intervention. These experiments will advance preclinical development of EIDD-1931/2801 towards IND submission.

A detailed description of the qualitative and quantitative criteria and data elements to be used to assess the scientific merit of feasibility of proceeding to the next stage of development

We have identified a number of key selection criteria that are considered critical for a broad-spectrum antiviral drug indicated for the treatment of SARS-CoV and MERS-CoV infections. We have shown that the [redacted] (b) (4) [redacted] (b) (4) designated EIDD-2801, meets a number of these criteria, and we are in the process of testing the drug against other criteria. An overview of our progress is described below.

1. *The compound provides broad-spectrum efficacy against SARS-CoV and MERS-CoV, and a resistance profile that does not promote the rapid emergence of escape mutants.* While a complete activity spectrum analysis has not yet been completed, [redacted] (b) (4)

   Based on in vitro efficacy data, we are confident that EIDD-1931/2801 will meet this efficacy criterion.

   A resistance profile for EIDD-1931/2801 has not yet been generated for coronaviruses. However, EIDD-1931 induces only minimal resistance in VEEV after 20 passages in the presence of drug, and this resistance resolves quickly upon drug free passaging (1). Because of findings observed with similar RNA viruses (e.g., VEEV, CHKV and IAV (data not shown)), we anticipate that we will see similar results against coronaviruses.

2. [redacted] (b) (4)

(b) (4)

[redacted]

(b) (4)

[redacted]

(b) (4)

[redacted]

1. *Virus fidelity* – we will determine the effect of EIDD-1931/2801 on viral RNA integrity as a potential mode of action through deep-sequencing, long-read sequencing, and determination of specific infectivity by qRT-PCR and plaque assay.
2. *Development of resistance* – we will determine the rate at which relevant CoVs develop resistance against EIDD-1931/2801 by passaging CoVs in the presence of increasing concentration of drug and monitoring resistance over time. Virus populations will be deep-sequenced at intervals to identify resistance-associated mutations. Mutations will be reverse-engineered into parental virus to define the impact of resistance on drug sensitivity and determine mechanism of action.
3. *Effect of resistance mutations on virulence and therapeutic efficacy*. We will infect murine models of MERS and SARS with EIDD-1931/2801-resistant mutants to determine the impact of drug resistance on CoV virulence and pathogenicity and the capacity of EIDD-1931/2801 to improve clinical outcomes.
4. *Impact of resistance mutations on sensitivity to other drugs.* We will determine the sensitivity of EIDD-1931/2801 resistant viruses to other drugs and assess the general fidelity cost of resistance.
5. *Combination therapy*
    i. Synergy and breadth of activity. We will perform *in vitro* experiments using continuous cell lines and cultured cells to determine if treatment with combinations of EIDD-1931/2801 with other direct-acting antivirals improve potency, prevent resistance, and increase breadth across genetically diverse CoVs.
    ii. *In vivo* efficacy and therapeutic window. We will infect murine models of MERS and SARS to determine if treatment with combinations of EIDD-1931/2801 with immunomodulators extends the therapeutic window for intervention.

Development Activities Covered By Contracts HDTRA1-C-15-0075 and HHSN272201500008C

A. Pharmacokinetics and Pharmacodynamics

1. *Pharmacokinetics and Tissue Distribution in Rats and Dogs* – The pharmacokinetic and tissue distribution profile of EIDD-1931/2801 will be determined to support subsequent toxicology studies and to provide a broadened basis for scaling among species to predict human exposure. Plasma levels of EIDD-1931 will be determined at three oral dose levels and one intravenous dose level. Tissues (lung, brain, spleen) will be collected 1 – 2 hours after C_{max} to determine levels of the [redacted] (b) (4) of EIDD-1931. No issues here would result in discontinuation of development.

B. Toxicology – All studies below are critical to the advancement of EIDD-2801, and significant and clinically relevant toxicity in any of these studies could result in a No-Go decision.

1. *Non-GLP DRF Studies* – We plan to conduct these studies in rodent (rats) and non-rodent (dogs) species to inform dosing regimens in subsequent 28-day toxicology studies. The dosing regimen used for the DRF studies will be based upon *in vivo* efficacy data and pharmacokinetic data. The studies will be conducted in 2 phases: 1) an escalating acute dosing phase to aid in the selection of doses for the next phase, and 2) a multiple (7-day) dose phase that will assess three dose levels. Toxicokinetics will be evaluated as well as clinical signs, blood chemistry and organ weights. These studies will serve as Go/No-Go gates for EIDD-2801. Justification for a No-Go decision to GLP toxicokinetic studies would include dose limiting toxicity at exposure levels that are 3-fold less than the efficacious AUC in mice.
2. *Definitive Genetic Toxicology Studies* – The potential of EIDD-2801 to cause genetic toxicity will be assessed by conducting *in vitro* studies: Bacterial Reverse Mutation Assay, *In Vitro* Mammalian Chromosome Aberration Test, and if necessary the Mammalian Erythrocyte Micronucleus Test. These studies will be run in parallel with the GLP 28-day toxicology studies.
3. *GLP Safety Pharmacology* – The potential of EIDD-2801 to cause adverse events in specific biological systems will be assessed, as required for a clinical drug candidate. Safety pharmacology studies will include tests for potential CNS (functional observational battery [FOB] study in rats), cardiovascular (cardiac safety pharmacology study in telemetered beagle dogs and *in vitro* hERG blockade assessment) and pulmonary (standard respiratory parameters in rats) effects. These studies will be run in parallel with the GLP 28-day toxicology studies.
4. *GLP Toxicology Studies* – IND-enabling studies are planned in rodent (rats) and non-rodent (dogs) species. The duration of these investigations is driven by the duration of dosing required for the proposed indications, which is expected to be no more than 10 to 14 days for acute RNA virus infections. We plan to conduct 28-day sub-chronic general toxicology studies to support the FIH clinical study. Each study will consist of 4 dosing groups (a control group and 3 drug dose groups). The doses and dosing regimen

will be determined by data from *in vivo* pharmacology and DRF toxicology studies. Gross necropsy and histopathology will be conducted on all animals. A 28-day recovery group will be included in the study. The NOAEL calculated from the more sensitive of the two species will be used to determine the initial FIH starting dose using methods described in the FDA guidance document (Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, FDA, CDER, July 2005).

C. Chemistry, Manufacturing and Controls – Synthesis and physiochemical properties support Go. No unresolvable issues are anticipated.

1. *cGMP Synthesis of Toxicology and Clinical Materials* - Initial scale-up operations have been carried out with EIDD-2801 and the material has been prepared in greater than 500g scale. The resulting drug substance meets our current draft release specifications which include: 1. Purity >95% (HPLC, elemental analysis), 2. No single impurity >0.5%, 3. Residual solvents – as per ICH Guidelines, 4. XRPD indicates a single polymorph. We believe the synthesis to be suitable for scale-up and of sufficient quality to move into process development for the production of toxicology and clinical supplies. The current plan calls for making drug substance for both animal toxicology and clinical studies in a single batch in order to eliminate the need for batch-to-batch qualifications.
2. *Drug Substance Stability* - A controlled stability program will be carried out according to FDA guidelines to characterize the stability of the drug substance under both ambient and accelerated conditions. This work is anticipated to be included as part of cGMP process development.
3. *Preformulation and Formulation Development for Toxicology* - Standard preformulation studies will be performed to determine the physiochemical properties that impact development of a dosing formulation to support *in vivo* efficacy, pharmacokinetic and toxicology studies. This data will also support the development of a drug product suitable for use in the FIH study.
4. *Drug Product Development for Early Clinical Trials* - A clinical dose formulation suitable for early clinical studies will be identified and the resulting drug product will be manufactured. A controlled stability program will also be carried out to characterize the stability of the drug product at both ambient and accelerated conditions. We anticipate that the initial drug product will be a (b) (4)

5. *The performance specs/features the product should have in order to provide therapeutic benefit*

Critical properties, which include oral bioavailability, efficient tissue distribution and anabolism, an acceptable safety profile, and good efficacy in animal models of RNA viral disease, have been outlined in section 4 above. EIDD-1931/2801 has met all Go/No-Go criteria to advance to clinical development status under contracts HDTRA1-15-C-0075 and HHSN272201500008C.

6. *A description and developmental status of the assays for the product release and characterization, including activity and efficacy (this may not be needed per se; depends on the stage of drug development)*

All reagents, assays and study protocols required for the nonclinical development of EIDD-1931/2801 are in place either at The Emory Institute for Drug Development or at the Contract Research Organizations currently identified for the development of EIDD-1931/2801. Similarly, all assays and reagents needed for the proposed characterization of EIDD-1931/2801 for the treatment of coronavirus infections are known and in place except for resistant viral mutants against EIDD-1931, which will be generated during the course of the program.

7. *Discussion with the FDA, if any, relevant to the development activities of the candidate product.*

No formal communications have occurred with the Food and Drug Administration (FDA) to date regarding the development of EIDD-1931/2801. A formal pre-IND meeting will be requested with the Agency before carrying out pivotal 28-day toxicology studies.

Cited References

1. Urakova N, Kuznetsova V, Crossman DK, Sokratian A, Guthrie DB, Kolykhalov AA, et al. β-d-N4-Hydroxycytidine Is a Potent Anti-alphavirus Compound That Induces a High Level of Mutations in the Viral Genome. J Virol. 2018 Feb 1;92(3):e01965-17.
2. Popowska E, Janion C. N4-hydroxycytidine-a new mutagen of a base analogue type. Biochem Biophys Res Commun. 1974 Jan 23;56(2):459–66.
3. Haynes P, Lambert TR, Mitchell ID. Comparative in-vivo genotoxicity of antiviral nucleoside analogues; penciclovir, acyclovir, ganciclovir and the xanthine analogue, caffeine, in the mouse bone marrow micronucleus assay. Mutat Res. 1996 Jul 10;369(1–2):65–74.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Prof. First Name*: Mark Middle Name R Last Name*: Denison Suffix: M.D.

Position/Title*: Craig-Weaver Professor of Pediatrics
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Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2581

Phone Number*: 6153222250 Fax Number:

E-Mail*: mark.denison@vanderbilt.edu

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: M.D. Degree Year: 1980

Attach Biographical Sketch*: File Name: Biosketch-Denison_3-14-18.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name (b) Last Name*: (b) (3) (A), (b) (6) Suffix: Ph.D

Position/Title*: Research Assistant Professor of Pediatrics
Organization Name*: Vanderbilt University Medical Center
Department: Pediatrics
Division:
Street1*: 1161 21st Avenue S., (b) (4), (b) (3) (A) Medical Center North
Street2:
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2581

Phone Number*: (b) (3) (A), (b) (6) Fax Number: Ardina

E-Mail*: ardina.(b) (3) (A), (b) (6)

Credential, e.g., agency login:

Project Role*: Faculty Other Project Role Category:

Degree Type: Ph.D. Degree Year: 2008

Attach Biographical Sketch*: File Name: Biosketch-(b) (3) (A), (b) (6)_3-6-18.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name* (b) (3) (A), (b) (6) Middle Name (b) (Last Name*: (b) (3) (A), (b) (6) Suffix: M.D.

Position/Title*: Research Associate Professor of Pediatrics
Organization Name*: Vanderbilt University Medical Center
Department: Pediatrics
Division:
Street1*: 1161 21st Avenue S.
Street2: (b) (4), (b) (3) (A) Medical Center North
City*: Nashville
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2581

Phone Number* (b) (3) (A), (b) (6) Fax Number:

E-Mail* (b) (3) (A), (b) (6)

Credential, e.g., agency login:

Project Role*: Faculty Other Project Role Category:

Degree Type: M.D., Ph.D. Degree Year: 2001, 1997

Attach Biographical Sketch*: File Name: Biosketch-(b) (3) (A), (b) (6)_3-9-18.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix: Ph.D

Position/Title*: Director
Organization Name*: (b) (3) (A), (b) (6)
Department: Biology
Division:
Street1*: 333 Lakeside Drive
Street2:
City*: Foster City
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 94404-1147

Phone Number*: ((b) (3) (A), (b) (6) Fax Number:
E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login:

Project Role*: Consultant Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1992

Attach Biographical Sketch*: File Name: Biosketch-(b) (3) (A), (b) (6).pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Professor
Organization Name*: The University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: 2107 McGavran Greenberg Hall
Street2: Campus Box #7435
City*: CHAPEL HILL
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (b) (3) (A), (b) (6) Fax Number:
E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1982

Attach Biographical Sketch*: File Name: (b) (3) (A), (b) (6)
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Professor
Organization Name*: The University of North Carolina at Chapel Hill
Department: Microbiology and Immunology
Division: School of Medicine
Street1*: Rm 22-006 Lineberger Cancer Ctr.
Street2: Campus Box 7295
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7295

Phone Number*: (b) (3) (A), (b) (6) Fax Number:
E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1975

Attach Biographical Sketch*: File Name: Bio_(b) (3) (A), (b) (6).pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (Middle Name Last Name*: (b) (3) (A), (b Suffix:

Position/Title*: Research Associate Professor
Organization Name*: The University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: 3004 Michael Hooker Research Bldg
Street2: Campus Box 7435
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (b) (3) (A), (b) (6) Fax Number:
E-Mail* (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2001

Attach Biographical Sketch*: File Name: Bio(b) (3) (A), (b) (6.pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Research Assistant Professor
Organization Name*: The University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: 3109 Michael Hooker Research Bldg
Street2: Campus Box 7435
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (b) (3) (A), (b) (6) Fax Number:

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2008

Attach Biographical Sketch*: File Name: (b) (3) (A), (b) (6)

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name* (b) (3) (A), (b) (6) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Research Assistant Professor
Organization Name*: The University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: 3109 Michael Hooker Research Bldg
Street2: Campus Box 7435
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (b) (3) (A), (b) (6) Fax Number:

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2006

Attach Biographical Sketch*: File Name: Bios_(b) (3) (A), (b) (6).pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix:	First Name*: (b) (3) (A), (b) (6)	Middle Name	Last Name*: (b) (3) (A), (b)	Suffix:

Position/Title*:	Research Associate
Organization Name*:	The University of North Carolina at Chapel Hill
Department:	Lineberger Compr Cancer Center
Division:	
Street1*:	450 W Drive , Suite 10-000
Street2:	Campus Box 7295
City*:	Chapel Hill
County:	Orange
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27599-7295

Phone Number*: (b) (3) (A), (b) (6)	Fax Number:
E-Mail*: (b) (3) (A), (b) (6)	
Credential, e.g., agency login: (b) (6)	
Project Role*: Other Professional	Other Project Role Category: Research Associate
Degree Type: MD	Degree Year: 2008

Attach Biographical Sketch*:	File Name:	Bio(b) (3) (A), (b) (6.pdf
Attach Current & Pending Support:	File Name:	

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Mark		Denison		Principal Investigator		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,227,383.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,227,383.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,227,383.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost (1st 25,000 of 2 subawards)	48.5	50,000.00	24,250.00
		Total Indirect Costs	**24,250.00**

Cognizant Federal Agency DHHS , Shon Turner, 214 767-3261
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,251,633.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,251,633.00

L. Budget Justification* File Name:
Budget_Justification_Corona_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Mark		Denison		Principal Investigator	(b) (6), (b) (4)	(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,248,560.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,248,560.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,248,560.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	48.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,248,560.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,248,560.00

L. Budget Justification* File Name:
Budget_Justification_Corona_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Mark		Denison		Principal Investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,245,724.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,245,724.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,245,724.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	48.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,245,724.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,245,724.00

L. Budget Justification*

File Name:
Budget_Justification_Corona_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Mark		Denison		Principal Investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,250,887.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,250,887.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,250,887.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	48.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,250,887.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,250,887.00

L. Budget Justification*	File Name: Budget_Justification_Corona_Overall.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Mark		Denison		Principal Investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	0.00
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	0.00

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,248,255.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,248,255.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,248,255.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	48.5	0.00	0.00
		Total Indirect Costs	**0.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,248,255.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,248,255.00

L. Budget Justification*	File Name: Budget_Justification_Corona_Overall.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION PROJECT 1 CORONAVIRUS

UAB has the prime CETR award; the Administrative Core will administer the subawards to the two project sites involved with Project 1. Please find the budget justification for those sites under their respective subaward budgets.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		6,220,809.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	6,220,809.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		6,220,809.00
Section H, Indirect Costs		24,250.00
Section I, Total Direct and Indirect Costs (G + H)		6,245,059.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		6,245,059.00

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Mark		Denison		Project Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			28,050.00	3,562.00	31,612.00
2.		[redacted]	[redacted]	(b) (6), (b) (3) (A) [redacted]		Co-Investigator	[redacted]	[redacted]			12,753.00	2,768.00	15,521.00
3.		[redacted]	[redacted]	[redacted]		Co-Investigator	[redacted]	[redacted]			44,483.00	9,653.00	54,136.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **101,269.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical	(b) (6) [redacted]					
3	Sr. Research Specialist	[redacted]			69,064.00	18,370.00	87,434.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**87,434.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**188,703.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1. TECAN HP D300e Digital Dispenser	41,020.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**41,020.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	70,177.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. CORES Sequencing	30,500.00
9. Computer software and FedEx expenses	1,600.00
10. Repairs/Maintenance	11,000.00
Total Other Direct Costs	**116,277.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**350,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	58.0	309,980.00	179,208.00
		Total Indirect Costs	**179,208.00**

Cognizant Federal Agency HHS: Steve Zuraf (301-492-4855)
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**529,208.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	529,208.00

L. Budget Justification* File Name:
VUMC_Budget_Justification_FINAL_3-14-18.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Mark		Denison		Project Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			28,050.00	3,562.00	31,612.00
2.		(b) (6), (b) (3) (A) [redacted]				Co-Investigator	[redacted]	[redacted]			13,008.00	2,823.00	15,831.00
3.		[redacted]				Co-Investigator	[redacted]	[redacted]			45,373.00	9,846.00	55,219.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **102,662.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Sr. Research Specialist	(b) (6) [redacted]			70,445.00	18,738.00	89,183.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**89,183.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**191,845.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Vanderbilt University Medical Center
Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	86,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. CORES Sequencing	42,500.00
9. Computer software and FedEx expenses	2,000.00
10. Repairs/Maintenance	15,000.00
Total Other Direct Costs	**150,500.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**348,345.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	58.0	348,345.00	202,040.00
		Total Indirect Costs	**202,040.00**

Cognizant Federal Agency HHS: Steve Zuraf (301-492-4855)
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**550,385.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	550,385.00

L. Budget Justification*
File Name:
VUMC_Budget_Justification_FINAL_3-14-18.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Mark		Denison		Project Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			28,050.00	3,562.00	31,612.00
2.	[redacted]	[redacted]	[redacted]	(b) (6), (b) (3) (A) [redacted]	[redacted]	Co-Investigator	[redacted]	[redacted]			13,269.00	2,879.00	16,148.00
3.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	Co-Investigator	[redacted]	[redacted]			46,280.00	10,043.00	56,323.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **104,083.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Sr. Research Specialist	(b) (6) [redacted]			71,854.00	19,113.00	90,967.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**90,967.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**195,050.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	86,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. CORES Sequencing	37,500.00
9. Computer software and FedEx expenses	2,000.00
10. Repairs/Maintenance	15,000.00
Total Other Direct Costs	**145,500.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**346,550.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	58.0	346,550.00	200,999.00
		Total Indirect Costs	**200,999.00**

Cognizant Federal Agency HHS: Steve Zuraf (301-492-4855)
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**547,549.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	547,549.00

L. Budget Justification* File Name:
VUMC_Budget_Justification_FINAL_3-14-18.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Mark		Denison		Project Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			28,050.00	3,562.00	31,612.00
2.		[redacted]	[redacted]	(b) (6), (b) (3) (A) [redacted]		Co-Investigator	[redacted]	[redacted]			13,534.00	2,937.00	16,471.00
3.		[redacted]	[redacted]	[redacted]		Co-Investigator	[redacted]	[redacted]			47,205.00	10,244.00	57,449.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **105,532.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Sr. Research Specialist	(b) (6) [redacted]			73,291.00	19,495.00	92,786.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**92,786.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**198,318.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Vanderbilt University Medical Center
Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	86,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. CORES Sequencing	37,500.00
9. Computer software and FedEx expenses	2,000.00
10. Repairs/Maintenance	15,000.00
Total Other Direct Costs	**145,500.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**349,818.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	58.0	349,818.00	202,894.00
		Total Indirect Costs	**202,894.00**

Cognizant Federal Agency HHS: Steve Zuraf (301-492-4855)
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**552,712.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	552,712.00

L. Budget Justification* File Name:
VUMC_Budget_Justification_FINAL_3-14-18.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Mark		Denison		Project Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			28,050.00	3,562.00	31,612.00
2.		[redacted]	[redacted]	(b) (6), (b) (3) (A) [redacted]		Co-Investigator	[redacted]	[redacted]			13,805.00	2,996.00	16,801.00
3.		[redacted]	[redacted]	[redacted]		Co-Investigator	[redacted]	[redacted]			48,150.00	10,447.00	58,597.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **107,010.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Sr. Research Specialist	(b) (6) [redacted]			74,757.00	19,885.00	94,642.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**94,642.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**201,652.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Vanderbilt University Medical Center
Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	86,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. CORES Sequencing	32,500.00
9. Computer software and FedEx expenses	2,000.00
10. Repairs/Maintenance	15,000.00
Total Other Direct Costs	**140,500.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**348,152.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Cost	58.0	348,152.00	201,928.00
		Total Indirect Costs	**201,928.00**

Cognizant Federal Agency HHS: Steve Zuraf (301-492-4855)
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**550,080.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	550,080.00

L. Budget Justification* File Name:
VUMC_Budget_Justification_FINAL_3-14-18.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

VUMC BUDGET JUSTIFICATION (PROJECT 1- (b) (3) (A), (b) (6)

PERSONNEL

Mark R. Denison, M.D. (Professor, (b) (6) effort, (b) (6) calendar months). Dr. Denison will serve as the Principal Investigator of Project 2. Dr. Denison has over 30 years of experience studying coronavirus replication and replicase nonstructural protein functions. He has published extensively on replication, molecular biology, protein functions and fidelity of coronaviruses. He has a long-standing, highly productive collaboration with Dr. Ralph Baric (18 years) for joint studies of the pathogenesis and inhibition of SARS-CoV and MERS-CoV, with strong synergy of directed mutagenesis and *in vitro* replication and biochemistry studies in the Denison lab and *in vivo* pathogenesis studies in the Baric Lab. Dr. Denison and (b) (3) (A), (b) (6) communicate directly on a weekly basis on this project. Dr. Denison will oversee these collaborations and will schedule conference calls and meetings. The Denison and (b) (3) (A), (b) (6) meet in person three times a year and have monthly phone calls for discussion of collaborative projects).

(b) (3) (A), (b) (6), Ph.D. (Research Assistant Professor, (b) (6) effort, (b) (6) calendar months). (b) (3) ((b) (3) (A), (b) (6) joined the Denison lab as Res. Asst. Prof. in Nov. 2017. (b) (3) (A), (has (b) (3) (A years of virology experience at BSL-2 and will carry a leading role in the laboratory antivirals program. Prior to joining the lab, (b) (3) (A), (b) (6) served as a project manager for the (b) (3) (A), (b) (6) (b) (3) (A), (b) will oversee and perform studies in Aims 1 and 2. (b) (3) (A), (b) (6) will be a BSL-3 / Select Agent investigator and will work with (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6), to perform studies at BSL-3 using MERS-CoV and SARS-CoV. Additionally, she will interact with collaborators on data analysis, presentation, and publication. (b) (3) (A), (will serve as the organizer of scientific data and discussions from the group, and interact with Program Managers at UNC, UAB, Emory, and (b) (3) (A), (b) (6).

(b) (3) (A), (b) (6), M.D., Ph.D. (Research Associate Professor, (b) (6) effort, (b) (6) calendar months). (b) (3) (A (b) (3) (A), (b) (6) is Res. Assoc. Prof. with over (b) (3) (A years of virology experience. He directs the (b) (3) (A), (b) (6) BSL-3 SARS-CoV and MERS-CoV research program. (b) (3) (A), (b) (6) is an authorized Select Agent user and will coordinate all investigators and studies in the BSL-3. He will oversee all experiments using engineered SARS-CoV and MERS-CoV in the BSL-3 and contribute advice on scientific direction and experimental strategy.

(b) (3) (A), (b) (6), M.S. (Senior Research Specialist, (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) has worked with Dr. Denison for over (b) (3) (years. (b) (3) (A), (b has extensive experience in performing mutagenesis and in engineering recombinant viruses using reverse genetics systems for SARS-CoV, and MERS-CoV. (b) (3) (A), (b) (6) will support experiments at BSL-2 for Aim 2, including preparation of mutagenized reverse-genetic plasmids, viral RNA sequencing, and data analysis. (b) (3) (A), (will coordinate all project purchases.

(b) (3) (A), (b) (6), M.S. (Senior Research Specialist, (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) joined the Denison lab in (b) (3) (A), (b) (6). Prior to her graduate studies, she worked several years in the pharmaceutical industry, engaged in multiple facets of drug development and product processing. (b) (3) (A), (b has extensive experience in biochemistry, molecular biology, microbiology, and *in vitro* cell culture systems. (b) (3) (A), (b) (6) is currently training for approval as a Select Agent user and will work with (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6) to passage MERS-CoV and SARS-CoV in the presence of drugs, identify resistance mutations, and perform quantitative testing of CoV-active lead compounds and structural analogues at BSL-3.

(b) (3) (A), (b) (6) (Senior Research Specialist, (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) has (b) (3) (A) years of experience with core high throughput sequencing and analysis of large data sets. (b) (3) (A), (will perform work supporting Aims 2 and 3, including MinION analysis, genomic analysis, and phenomic studies of drug-resistant MHV mutant viruses; assist (b) (3) (A), (b) (6) in the engineering of recombinant CoVs using reverse-genetics techniques; and preparare cell cultures and reagents for experiments performed at BSL-3. She also will focus on preparation, analysis and presentation of sequencing data to look at diversity of populations in wild-type and resistant isolates.

FRINGE BENEFITS: Fringe benefit calculations are derived (b) from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES (Year 1: $70,177; Years 2-5: $86,000)

Cell Culture Supplies (Year 1: $13,627; Years 2-5: $20,000). Funds are requested for media, serum, sterile plastic ware, and related cell culture supplies to maintain multiple cell lines and measure the effect of drugs on a variety of virus replication parameters by plaque assay and plate reader-based assays. We predict an increase in cell culture supply cost in years 2-5 based on the scope of work and the experimental timeline.

BSL-3 Supplies (Year 1: $16,000; Years 2-5: $20,000). All testing of drugs against SARS-CoV and MERS-CoV, including virus passaging, monitoring development of drug resistance, and confirmation of resistance to lead compounds, will be performed under strict BSL-3 protocols. This will include extensive use of plastic ware, tissue culture reagents, and materials for plaque assays and RNA isolation. BSL-3 PPE (personal protective equipment) is required for all work done at BSL-3. Materials for autoclaving and disposal of waste are also included in this category. Dedicated reagents are required for analysis of SARS-CoV RNA at BSL-2 because viral RNA is regulated as a Select Agent. We predict an increase in BSL-3 supply cost in years 2-5 based on the type and number of MERS- and SARS-CoV experiments proposed in those years.

Enzymes and Reagents (Years 1-5: $12,000). Engineering mutations within plasmids carrying fragments of viral genomes for the experiments proposed in Aims 2 and 3 will require enzymes and reagents specified by the corresponding protocols. Assembling recombinant MHV, SARS-CoV, and MERS-CoV requires large amounts of expensive restriction enzymes (e.g., BsmBI) and DNA ligase. Molecular biology grade agarose, DNA purification kits, and DNA markers are needed for identifying and isolating assembly intermediates to generate full-length cDNA clones of recombinant viruses. In addition, recovery of viruses from cloned cDNAs requires expensive T7 RNA polymerase-driven *in vitro* transcription kits and electroporation supplies for introduction of infectious full-length viral RNA transcripts into susceptible cells. Substantial amounts of real-time PCR supplies and reagents (e.g., reaction kits and fluorescent probes) are required to support experimental procedures proposed in Aims 2 and 3. Cytotoxicity kits (e.g., CellTiter-Glo) as well as the 96-well plates and other plastic ware will be used to examine the toxicity of drug combinations in Aim 3. Basic chemicals and buffers are needed for general laboratory techniques.

MinION sequencing (Years 1-5: $28,050). Experiments proposed in Aim 2 require specialized reagents for MinION library preparation and long-read single-molecule sequencing ($830/sample; estimated 34 samples/year).

CO2 and LN2 (Year 1: $500; Years 2-5: $1,000). Cell culture requires regular delivery of CO_2 for incubators, and cryopreservation of cell lines used in this project requires periodic deliveries of LN_2. We predict an increase in CO2 and LN@ cost in years 2-5 based on an increase in incubator and cryopreservation capacity needs as the experimental timeline progresses.

OTHER EXPENSES (Year 1: $46,100; Year 2: $64,500; Years 3-4: $59,500; Year 5: $54,500)

Sanger sequencing (Years 1-5: $2,500). Funds are requested for Di-deoxy (Sanger) sequencing of full viral genomes to identify mutations in Aim 2 and to confirm engineered mutations in genome fragments and potential reversion and compensating mutations in Aims 2 and 3.

Deep sequencing (Year 1: $28,000; Year 2: $40,000; Years 3-4: $35,000; Year 5: 30,000). Funds are requested towards deep sequencing of viral genomes to determine mutation rates and types induced by drug treatment. Cost of deep sequencing for year 1 is budgeted at 40 samples at $700/sample which includes amplicon construction by our laboratory followed by and library preparation and sequencing by the Vanderbilt Technologies for Advances Genomics (VANTAGE) core. We predict an increase in deep sequencing cost in years 2-5 based on the experimental timeline.

Computer supplies (Years 1-5: $1,000). Funds are requested for project specific computer and software upgrades required to analyze data and communicate results.

FedEx Shipping (Year 1: $600; Years 2-5: $1,000). Funds are requested to cover the costs of shipping samples to the Baric laboratory for analysis. We predict an increase in shipping cost in years 2-5 based on the expected increase in materials produced by each laboratory.

Repairs and Maintenance (Year 1: $11,000; Years 2-5: $15,000): This category includes maintenance of equipment and service contracts on major equipment in BSL-2 spaces as well as annual decontamination and complete recertification of the BSL-3 lab, which includes required preventative maintenance of equipment and service contracts on major equipment. The BSL3 decontamination and recertification includes replacement of HEPA filters, PM, and vapor phase H_2O_2 sterilization. The percent of cost for this proposal for each year is based on the number of ongoing BSL-3 grants / projects. At this time we propose 33% of cost for this process attributable to this proposal.

Publications (Year 1: $3,000; Years 2-5: $5,000): Sufficient for 1 publication per year. This is increased in out years as more publications are anticipated.

TRAVEL (Year 1: $4,000; Years 2-5: $6,000): Budgeted amount will allow partial support of travel for two investigators to attend one meetings per year for presentation of scientific results. Relevant venues include the International Congress on Antiviral Research (ICAR), and the American Society for Virology. In addition, these funds will allow travel for participating investigators to the University of Alabama at Birmingham (UAB) for the annual Centers for Excellence in Translational Research (CETR) symposium at UAB. This is increased in years 2-5 based on the potential international location of ICAR.

EQUIPMENT: (Year 1: $41,020)

TECAN HP D300e Digital Dispenser ($41,020): the digital dispenser will enable precise dilution and dispensing of drugs to the desired final concentration for the determination of EC_{50} values in Aim 1 and synergy experiments proposed in Aim 3. The ability to perform synergy studies requires complex, and highly reproducible dilutions within and across experiments, not achievable by multipipette or hand pipetting. This is requested in year 1 as it is critical for experiences throughout the proposed studies. For rapid movement to timelines and milestones associated with preclinical development, experiment for synergy and time of addition studies this is essential and currently unavailable technology for the (b) (3) (A), (b) (6)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		520,556.00
Section B, Other Personnel		455,012.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		975,568.00
Section C, Equipment		41,020.00
Section D, Travel		28,000.00
1. Domestic	28,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		698,277.00
1. Materials and Supplies	414,177.00	
2. Publication Costs	23,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	180,500.00	
9. Other 2	9,600.00	
10. Other 3	71,000.00	
Section G, Direct Costs (A thru F)		1,742,865.00
Section H, Indirect Costs		987,069.00
Section I, Total Direct and Indirect Costs (G + H)		2,729,934.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		2,729,934.00

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	(b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	(b) (6), (b) (4)	(b) (6)			18,960.00	5,212.00	24,172.00
2.		[redacted]	[redacted]	[redacted]		Investigator	[redacted]	[redacted]			9,480.00	2,606.00	12,086.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			24,472.00	7,143.00	31,615.00
4.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			22,385.00	6,928.00	29,313.00
5.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			21,961.00	6,824.00	28,785.00
6.		[redacted]	[redacted]	[redacted]	Ph.D	Research Associate	[redacted]	[redacted]			20,924.00	6,865.00	27,789.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **153,760.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	(b) (6)			7,875.00	1,787.00	9,662.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Research Special	[redacted]			29,759.00	9,606.00	39,365.00
1	Research Techni	[redacted]			16,000.00	6,250.00	22,250.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,277.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**225,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	2,500.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	144,636.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Deep sequencing;maintenance contracts	30,000.00
9. Histology; flow cytometry; animal per diem; MI Seq reactions; shipping	36,490.00
10. GRA Tuition	2,837.00
Total Other Direct Costs	**215,963.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**450,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MDTC	55.5	447,163.00	248,175.00
		Total Indirect Costs	**248,175.00**

Cognizant Federal Agency DHHS Division of Cost Allocation, Darryl Mayes 202 401-2808
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**698,175.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	698,175.00

L. Budget Justification* File Name:
UNC_Proj_1_Corona_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			18,960.00	5,212.00	24,172.00
2.		[redacted]	[redacted]	[redacted]		Investigator	[redacted]	[redacted]			9,480.00	2,606.00	12,086.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			24,472.00	7,143.00	31,615.00
4.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			22,385.00	6,928.00	29,313.00
5.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			21,961.00	6,824.00	28,785.00
6.		[redacted]	[redacted]	[redacted]	Ph.D	Research Associate	[redacted]	[redacted]			20,924.00	6,865.00	27,789.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **153,760.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	[redacted] (b) (6)					
1	Graduate Students	[redacted]			7,875.00	1,787.00	9,662.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Research Special	[redacted]			29,759.00	9,606.00	39,365.00
1	Research Techni	[redacted]			16,000.00	6,250.00	22,250.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,277.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**225,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	2,500.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	144,636.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Deep sequencing;maintenance contracts	30,000.00
9. Histology; flow cytometry; animal per diem; MI Seq reactions; shipping	36,490.00
10. GRA Tuition	2,837.00
Total Other Direct Costs	**215,963.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**450,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MDTC	55.5	447,163.00	248,175.00
		Total Indirect Costs	**248,175.00**

Cognizant Federal Agency DHHS Division of Cost Allocation, Darryl Mayes 202 401-2808
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**698,175.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	698,175.00

L. Budget Justification* File Name:
UNC_Proj_1_Corona_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Project Co-investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			18,960.00	5,212.00	24,172.00
2.		[redacted]	[redacted]	[redacted]		Investigator	[redacted]	[redacted]			9,480.00	2,606.00	12,086.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			24,472.00	7,143.00	31,615.00
4.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			22,385.00	6,928.00	29,313.00
5.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			21,961.00	6,824.00	28,785.00
6.		[redacted]	[redacted]	[redacted]	Ph.D	Research Associate	[redacted]	[redacted]			20,924.00	6,865.00	27,789.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **153,760.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b) (6) [redacted]					
1	Graduate Students	[redacted]			7,875.00	1,787.00	9,662.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Research Special	[redacted]			29,759.00	9,606.00	39,365.00
1	Research Techni	[redacted]			16,000.00	6,250.00	22,250.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,277.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**225,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: The University of North Carolina at Chapel Hill
Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	2,500.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	144,636.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Deep sequencing;maintenance contracts	30,000.00
9. Histology; flow cytometry; animal per diem; MI Seq reactions; shipping	36,490.00
10. GRA Tuition	2,837.00
Total Other Direct Costs	**215,963.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**450,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MDTC	55.5	447,163.00	248,175.00
		Total Indirect Costs	**248,175.00**

Cognizant Federal Agency DHHS Division of Cost Allocation, Darryl Mayes 202 401-2808
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**698,175.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	698,175.00

L. Budget Justification* File Name:
UNC_Proj_1_Corona_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2022 **End Date*:** 02-01-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Project Co-investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			18,960.00	5,212.00	24,172.00
2.		[redacted]	[redacted]	[redacted]		Investigator	[redacted]	[redacted]			9,480.00	2,606.00	12,086.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			24,472.00	7,143.00	31,615.00
4.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			22,385.00	6,928.00	29,313.00
5.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			21,961.00	6,824.00	28,785.00
6.		[redacted]	[redacted]	[redacted]	Ph.D	Research Associate	[redacted]	[redacted]			20,924.00	6,865.00	27,789.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **153,760.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b) (6) [redacted]					
1	Graduate Students	[redacted]			7,875.00	1,787.00	9,662.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Research Special	[redacted]			29,759.00	9,606.00	39,365.00
1	Research Techni	[redacted]			16,000.00	6,250.00	22,250.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,277.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**225,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: The University of North Carolina at Chapel Hill
Start Date*: 03-01-2022 **End Date*:** 02-01-2023 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	2,500.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2022 **End Date*:** 02-01-2023 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	144,636.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Deep sequencing;maintenance contracts	30,000.00
9. Histology; flow cytometry; animal per diem; MI Seq reactions; shipping	36,490.00
10. GRA Tuition	2,837.00
Total Other Direct Costs	**215,963.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**450,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MDTC	55.5	447,163.00	248,175.00
		Total Indirect Costs	**248,175.00**

Cognizant Federal Agency DHHS Division of Cost Allocation, Darryl Mayes 202 401-2808
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**698,175.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	698,175.00

L. Budget Justification* File Name:
UNC_Proj_1_Corona_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Project Co-investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			18,960.00	5,212.00	24,172.00
2.		[redacted]	[redacted]	[redacted]		Investigator	[redacted]	[redacted]			9,480.00	2,606.00	12,086.00
3.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			24,472.00	7,143.00	31,615.00
4.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			22,385.00	6,928.00	29,313.00
5.		[redacted]	[redacted]	[redacted]	Ph.D	Investigator	[redacted]	[redacted]			21,961.00	6,824.00	28,785.00
6.		[redacted]	[redacted]	[redacted]	Ph.D	Research Associate	[redacted]	[redacted]			20,924.00	6,865.00	27,789.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **153,760.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b) (6) [redacted]					
1	Graduate Students	[redacted]			7,875.00	1,787.00	9,662.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Research Special	[redacted]			29,759.00	9,606.00	39,365.00
1	Research Techni	[redacted]			16,000.00	6,250.00	22,250.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,277.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**225,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	2,500.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	144,636.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Deep sequencing;maintenance contracts	30,000.00
9. Histology; flow cytometry; animal per diem; MI Seq reactions; shipping	36,490.00
10. GRA Tuition	2,837.00
Total Other Direct Costs	**215,963.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**450,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MDTC	55.5	447,163.00	248,175.00
		Total Indirect Costs	**248,175.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) DHHS Division of Cost Allocation, Darryl Mayes 202 401-2808

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**698,175.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	698,175.00

L. Budget Justification* File Name:
UNC_Proj_1_Corona_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification Baric Project Whitley CETR 2018

Personnel:

(b) (3) (A), (b) (6)**, Ph.D., Principal Investigator** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) will supervise the overall direction of the animal research agenda of this highly interactive proposal. He will interact closely with Drs. Denison, (b) (3) (A), (b) (6), and (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6) to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. (b) (3) (A), (b) (6) will be also be responsible for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the (b) (3) (A), (b) (6), writing progress reports and managing fiscal matters associated with the proposal. (b) (3) (A), (b) (6) will work closely together to plan (b) (3) (A), (b) (4) experiments planned throughout the proposal. Given the extensive interaction and collaboration with Dr. Denison in the past, he will also lead efforts to coordinate and promote research efforts with the groups. (b) (3) (A (b) (3) (A), (b) (will communicate his findings with Dr. Denison on a regular basis via both conference calls and meetings between the two laboratories.

(b) (3) (A), (b) (6)**, Ph.D. Investigator** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) will oversee the (b) (3) (A), (b) (4) work within Project 1 to help define compound mechanism of action. (b) (3) (A), (b) (6) will work with (b) (3) (A), (b) (6) to oversee the work of(b) (3) (A), (b) (6). He will also work closely with the leadership team to coordinate efforts in his lab with those of the other members of the team. He will be responsible for all reporting for the work done in his lab to (b) (3) (A), (b) (6) and will contribute to the writing of scientific publications covering this work.

(b) (3) (A), (b) (6)**, Ph.D. Co-Investigator** (b) (6) effort, (b) (6) cal months)(b) (3) (A), (b) (6) has extensive select agent/BSL3 experience primarily working with in vitro models of the human conducting airway. (b) (3) (A), (has been performing drug testing in primary lung cell cultures for more than four years. (b) (3) (A), (b) (6) will perform all coronavirus drug testing in primary human cells to evaluate drug candidates/drug combinations in a more advanced human lung in vitro model. In addition, (b) (3) (A), will perform viral passage studies in the presence of drug candidates to identify escape mutations that may arise. Finally, (b) (3) (A), will oversee all select agent research in the facility. (b) (3) (A), (b) (6) will report findings regularly to (b) (3) (A), (b) (6) and Denison as well as interfacing with the drug candidate manufacturers.

(b) (3) (A), (b) (6)**, Ph.D. Investigator** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) has extensive BSL3 experience and is now a Research Assistant Professor within the (b) (3) (A), (b) (6). He will lead and perform the in vivo drug testing with wild type and resistant mutant strains of MERS-CoV experiments this project. He will be assisted by (b) (3) (A), (b) (6). He will consult with (b) (3) (A), (b) (6) and Denison regarding experimental design and analysis and will report findings regularly to the collaborative group as well as interfacing with the drug candidate manufacturers.

(b) (3) (A), (b) (6)**, Ph.D. Investigator** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) has extensive experience working with wild type SARS-CoV and SARS-ExoN I in mice and is skilled at assembling recombinant SARS-CoV viruses using classic recombinant DNA approaches or synthetic genomic approaches. (b) (3) (A), (b)will lead and perform the in vivo drug testing with wild type and resistant mutant strains of SARS-CoV for this project. (b) (3) (A), (will be assisted by (b) (3) (A), (b) (6). (b) (3) (A), (b)will consult with (b) (3) (A), (b) (6) and Denison regarding experimental design and analysis and will report findings regularly to the collaborative group as well as interfacing with the drug candidate manufacturers.

(b) (3) (A), (b) (6)**, Ph.D. Research Associate** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) will directly supervise (b) (3) (A. (b) (3) (A), (b) (in the creation of deep sequencing libraries. (b) (3) (A), (b) (6) will also be responsible for data analysis, and he will convey the results to other members of the research team. He will contribute, with (b) (3) (A), (b) (6), in all writing and reporting. (b) (3) (A), (b) (6) has worked over the last (b) (3) (A), years to bring the (b) (3) (A), (b) (4) into routine practice and is the ideal person to guide and analyze the proposed sequencing work to characterize mutation rates using deep sequencing linked to high accuracy data acquisition.

(b) (3) (A), (b) (6)**, Graduate Student** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) is a third year graduate student in the (b) (3) (A), (b) (6) who has recently gained independent access to the BSL3 laboratory. He will work with (b) (3) (A), (b) (6) to determine compound efficacy in SARS-CoV infected mice. He will consult with (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6) regarding experimental design and analysis and will report findings regularly to the collaborative group.

(b) (3) (A), (b) (6). **Research Specialist** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) has over (b) (3) (years of BSL3 experience and pioneered the infectious clone platforms used in the (b) (3) (A), (b) (6). He used synthetic DNAs to construct the SARS-CoV and MERS-CoV molecular clones and isolated numerous recombinant viruses. He is also well versed in performing routine plaque assays and at viral neutralization assays. (b) (3) (A), (b) (6) will assist (b) (3) (A), (b) (6) with in vivo MERS-CoV drug testing. He will report his results to (b) (3) (A), (b) (6) at regular intervals and prepare reports. (b) (3) (A), (b) (6) will also be responsible for purchasing supplies, maintaining stocks in the BSL3 and assisting with day to day maintenance of the BSL3 laboratory.

(b) (3) (A), (b) (6), **Research Specialist** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal infections and husbandry. (b) (3) (A), (will support (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6) research efforts as needed and will specifically assist (b) (3) (A), (b) (6) with all SARS-CoV in vivo drug testing. (b) (3) (A), (will also work with (b) (3) (A), (b) (6) to maintain BSL3 stocks and in day to day maintenance of the BSL3 laboratory.

(b) (3) (A), (b) (6), **Research Technician** (b) (6) effort, (b) (6) cal months). (b) (3) (A), (b) (6) has spent the last 18 months making deep sequencing libraries using the (b) (3) (A), (b) (4) in the (b) (3) (A), (b) (6), thus she is highly skilled to work with (b) (3) (A), (b) (6) in generating the data described in this proposal.

Fringe Benefits: Faculty/Staff: 23.293% Social Security and Retirement; $5,869/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,318/FTE Health Insurance.

Travel

Travel: ($9,000/year): Domestic Travel: Funds are requested for the Project Leader and staff to attend 2 scientific conferences and the annual CETR U19 meeting in Birmingham and the reverse site visits in Washington DC each year. This allows program faculty and fellows to communicate results, develop collaborations and share research interests. International Travel: Funds are requested to allow team members to attend the annual International Conference on Antiviral Research (ICAR) meeting to present current data and renewal and establish collaborations. (b) (3) (A), (b) (6) requested $6,000 in travel funds and (b) (3) (A), (b) (6) group $3,000.

Supplies:

Synthetic DNA ($8,500/year) Funds are requested for the purchase of synthetic DNA fragments which are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about $0.35/base. Our budget allows for ~30,000-bp of synthetic gene synthesis/yr, sufficient for our needs over the course of this project and will allow for the rapid assembly of recombinant viruses bearing different ExoNI derived mutations.

BL3 Protective Gear ($8,000/year) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Miscellaneous ($6,636/year) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings. (b) (3) (A), (b) (6) group requests $4,097 a year and (b) (3) (A), (b) (6) group $2,539 a year.

MiSeq Reagents ($13,000/year) Funds are requested for the (b) (3) (A), (b) (6) laboratory to purchase tubes, tips, PCR and indexing reagents to generate five library pools (cDNA synthesis, PCR, primers, indexing kits) to perform Primer ID.

Computer Supplies ($1,000/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

Primary Human Lung Cell Cultures ($50,000/year) Funds are requested to purchase mature human airway epithelial cell (HAE) cultures, primary human lung microvascular endothelial cells and fibroblasts for drug testing assays. Each culture is $125. We anticipate requiring 400 HAE cultures annually.

Molecular Biology Reagents ($10,000/year) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic acid markers. As sequence confirmation is critical prior to assembly of full length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of ExoNI mediated mutations.

Immunology Reagents ($8,000/year) Funds to purchase supplies for flow cytometry and ELISA based immune assays are requested to determine how drug treatment/time of addition studies modify the host immune response following CoV infection.

Tissue Culture Reagents ($13,000/year) We are requesting funds to purchase cell culture supplies and plastic ware to perform cell passage and viral plaque assays to determine titer reduction for drug efficacy studies.

Animals ($26,500/year) Funds are requested to purchase ~96 each- SCID ($68), RAG ($124), young BalbC ($25), aged BalbC ($20), young B6 ($20) and aged B6 ($18) mice at the indicated prices per mouse. These monies are essential for evaluating drug efficacy across hosts of differing susceptibilities to lethal infection.

Other Expenses:

Deep Sequencing ($25,000/year) The ExoN mutator phenotype results in high mutation rates which must be accessed by ultra deep sequencing methods like RNAseq, including informatics support to analyze the data. This also includes funds for supplies to generate amplicon library and to prepare the library for sequencing. As such, we anticipate significant sequencing costs over the duration of this proposal. The allotted budget will allow us to work with UNC's Vironomics Core facility and to be able to sequence approximately 45 samples (~20 samples $10,000) per year.

Publication Costs ($2,000/year) Funds are requested to cover the publication of manuscripts.

Maintenance Contracts ($5,000/year) Several instruments in the Baric Laboratory that will be used in these studies (centrifuges, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology ($9,000/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Flow Cytometry ($8,000/year) UNC-Chapel Hill provides a core facility with advanced analytical cytometers that can resolve >6 colors at a time, which is needed when delineating subsets of inflammatory cells following infection.

Animal per diem ($8,869/year) SCID, RAG and the young BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~30 days prior to the start of experiments (5 animals per cage x 30 days x 0.7 per cage). The aged BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~90 days prior to the start of experiments (5 animals per cage x 90 days x 0.7 per cage). All MERS-CoV 288/330 mice will be bred in house (~$4879/year).

MiSeq Deep Sequencing Runs ($10,000/year) Funds are requested for the Swanstrom laboratory to perform 5 MiSeq deep sequencing runs per year (300 bas paired-end reads) to perform Primer ID.

Shipping ($621/year) Funds are requested to cover the costs of shipping samples/viruses to the (b) (3) (A), (b) (6) (b) (3) (A), (b) (6) for analysis over the course of the proposal.

Tuition ($2,837/year) Funds are requested to cover 25% of Mr. Dinnon's tuition costs.

Indirect Costs (Facilities and Administration): In accordance with the University's F&A Cost Rate Agreement dated November 23, 2016, the University's predetermined F&A rate is 55.50%. This rate remains in effect through June 30, 2020. The base for calculating Facilities and Administrative cost (indirect cost) is modified total direct costs (MTDC), consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel and subcontracts up to $25,000 of each subcontract. Modified total direct costs shall exclude equipment, GRA tuition, rental costs of off-site facilities, scholarships, and the portion of each subcontract in excess of $25,000.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		768,800.00
Section B, Other Personnel		356,385.00
Total Number Other Personnel	20	
Total Salary, Wages and Fringe Benefits (A+B)		1,125,185.00
Section C, Equipment		0.00
Section D, Travel		45,000.00
1. Domestic	32,500.00	
2. Foreign	12,500.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		1,079,815.00
1. Materials and Supplies	723,180.00	
2. Publication Costs	10,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	150,000.00	
9. Other 2	182,450.00	
10. Other 3	14,185.00	
Section G, Direct Costs (A thru F)		2,250,000.00
Section H, Indirect Costs		1,240,875.00
Section I, Total Direct and Indirect Costs (G + H)		3,490,875.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		3,490,875.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Project_1_Specific_Aims_3-23-18.pdf
3. Research Strategy*	Project_1_CoV_Research_Strategy_3-26-18.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_Corona.pdf
6. Select Agent Research	Select_Agent_Corona.pdf
7. Multiple PD/PI Leadership Plan	U19-Whitley_Multiple_PI_Leadership_Plan_FINAL_3-14-18.pdf
8. Consortium/Contractual Arrangements	
9. Letters of Support	JORDAN_LOS_Denison_CETR_20180315.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_Corona.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_Corona.pdf
Appendix	
12. Appendix	

SPECIFIC AIMS

Zoonotic coronaviruses (CoVs) such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV are pandemic threats, with MERS-CoV continuing to cause new zoonotic and human transmission and illness, resulting in ~35% mortality. Currently, there are no FDA-approved therapies to treat any CoV. Several challenges for anti-CoV drug development exist. 1) As with SARS-CoV and MERS-CoV, new zoonotic CoVs likely will emerge from divergent virus pools in animal reservoirs, thus requiring antiviral strategies aimed at conserved targets. 2) Although nucleoside analogues/mutagens are effective against many RNA viruses by acting against viral RNA-dependent RNA polymerases, CoVs uniquely encode a novel RNA-dependent proofreading exoribonuclease (nsp14-ExoN), thus requiring effective nucleoside analogues to inhibit or resist the action of ExoN. 3) CoVs are able to rapidly develop resistance to multiple classes of inhibitors, demonstrating the need for approaches to prevent resistance emergence. 4) Both SARS and MERS progress to severe immunopathologic damage, potentially limiting the therapeutic window for direct-acting antivirals (DAAs). Thus, combinations of DAAs and targeted immunomodulators may be necessary for effective treatment of established infection.

The overall goal of our program is to develop CoV antiviral strategies that broadly inhibit known and future potential pandemic zoonotic CoVs, prevent emergence of resistance, and extend the therapeutic window by targeting host immunopathologic responses. Our previous work identified nucleoside analogues (NAs) and non-nucleoside compounds with potent activity against CoVs. Two lead NAs in advanced stages of preclinical development, remdesivir (GS-5734, Gilead Sciences) and EIDD-1931/2801 (Emory Institute for Drug Development), inhibit multiple CoVs, including SARS-CoV and MERS-CoV, in the presence of the intact ExoN. Other promising NAs are also in the pipeline. Two hits from screening at Southern Research (SR), SR-36097 and SR-35293, dramatically inhibit SARS-CoV replication in cell monoculture and in primary human airway epithelial cell cultures. The proposed research will advance preclinical development of the CoV-inhibitory EIDD and SR compounds for treatment and prevention of epidemic and pre-emergent CoVs, generating mechanistic and efficacy data necessary for IND filing and origination of human clinical trials. In addition, we will explore additive or synergistic effects of DAAs. Finally, we will establish proof-of-concept testing for combination of DAAs and immunomodulatory drugs to extend the therapeutic window and treat established infection *in vivo*. The impact of our program is high. Advancing new leads, increasing potency, broadening activity spectrum, expanding the therapeutic window, and preventing resistance of antiviral treatments for epidemic CoVs addresses an immediate unmet medical need for MERS-CoV and could prove to be an invaluable tool to prevent future pandemics due to pre-emergent CoVs and other high-consequence viral pathogens.

Aim 1. To determine the spectrum of antiviral activity and therapeutic efficacy of EIDD-1931/2801 and perform hit-to-lead stage evaluation of SR compounds. In this aim, we will (i) determine EIDD-1931 and other DAA activity spectra across CoV groups and genera; (ii) determine antiviral efficacy, metabolism, and cytotoxicity in human lung cells targeted by SARS- and MERS-CoV; (iii) evaluate the efficacy of EIDD-1931 and other DAA NA in young, aged, and immunosuppressed murine models of MERS and SARS pathogenesis; and (iv) perform hit-to-lead studies for SR compounds. SR compounds that meet all Go/No-Go criteria will be entered as lead compounds into the development pipeline as described for EIDD-1931/2801.

Aim 2. To define mechanism of action of EIDD-1931/2801 and impact of resistance mutations on *in vivo* therapeutic efficacy, viral fitness, and pathogenesis. In this aim, we will (i) define lead compound mechanisms of action by assessing the impact on CoV replication, chain termination, fidelity, and innate immune responses; (ii) assess the kinetics of drug-resistance development and impact of drug resistance on viral replication and fitness; (iii) determine the effect of drug resistance on sensitivity to other drugs; (iv) determine the impact of resistance mutations on MERS-CoV and SARS-CoV replication, fitness, and pathogenicity in mice; and (v) determine the impact of resistance mutations on therapeutic efficacy. Data collected in Aims 1 and 2 are crucial prerequisites to progression of lead compounds to clinical evaluation.

Aim 3. To test combination regimens for treatment of emerging CoV disease. Emerging coronaviruses evolve quickly and can cause acute severe end-stage immunopathologic lung disease (ARDS), which potentially limits therapeutic windows. Thus, new strategies are needed to extend the effective therapeutic window of DAAs. In this aim, we will (i) assess the combined efficacy and potency of DAAs against MERS-CoV and SARS-CoV *in vitro*; (ii) evaluate therapeutic and prophylactic efficacy of combined DAAs in murine models of MERS and SARS-induced ARDS; and (iii) determine the therapeutic efficacy of co-administered DAAs and immunomodulators in MERS- and SARS-CoV-infected mice. The impact of these studies is high; investigating new strategies to extend the therapeutic window of DAAs while reversing ARDS disease pathology is broadly relevant to many acute, highly pathogenic respiratory virus infections.

RESEARCH STRATEGY

A. SIGNIFICANCE AND IMPACT. Potent, broadly effective, resistance-averting therapies are urgently needed to protect against emerging, highly pathogenic zoonotic coronaviruses (CoVs), whose genetic diversity can render vaccines and therapeutics ineffective. There currently are no FDA-approved therapeutics for human or zoonotic CoVs. Our goal is to address this vulnerability by progressing CoV drug candidates toward an investigational new drug (IND) application.

Coronavirus pandemic potential, endemic human disease, and need for therapeutics. CoVs are a genetically diverse family of RNA viruses that infect a wide variety of mammalian and avian species. Many CoVs have demonstrated zoonotic capacity or potential[1–4]. While infection with endemic human CoVs (HCoVs) NL63, OC43, 229E and HKU1 typically causes self-limiting upper respiratory tract illnesses, infection of vulnerable individuals such as young children, the elderly, and immunocompromised individuals can cause significant morbidity and mortality[5–10]. The epidemics of severe acute respiratory syndrome (SARS)- and Middle East respiratory syndrome (MERS)-CoVs in humans has confirmed the capacity for CoVs to cause severe human disease and continued emergence[1–4]. In 2002, SARS-CoV emerged from bats in Guangdong China, causing over 8,000 cases with 10-50% mortality as a function of increasing age[11–15]. While SARS-CoV is not a current threat, SARS-like bat CoVs are currently circulating in nature that utilize the human SARS-CoV receptor (i.e., angiotensin converting enzyme 2, ACE2) for infection, replicate efficiently in primary human airway cells, and are resistant to existing pre-clinical therapeutic antibodies and vaccines[16–19]. In 2012, MERS-CoV was discovered to have evolved from bats to infect humans via intermediate camel hosts. MERS-CoV continues to cause illness and death (~35% mortality rate), with over 2,145 confirmed cases in at least 27 countries[1,20,21]. MERS-CoV is endemic and widespread among dromedary camel populations in the Arabian Peninsula and portions of Africa. MERS-CoV infections in camels are typically mild[22], creating a situation for continuing and unrecognized human exposures. As with SARS-CoV, viruses similar to MERS-CoV have been isolated from bats in China, Korea, Africa, and elsewhere[23–25]. With increasing overlap of human and wild animal ecologies, the potential for future severe zoonotic CoV emergence is high[26].

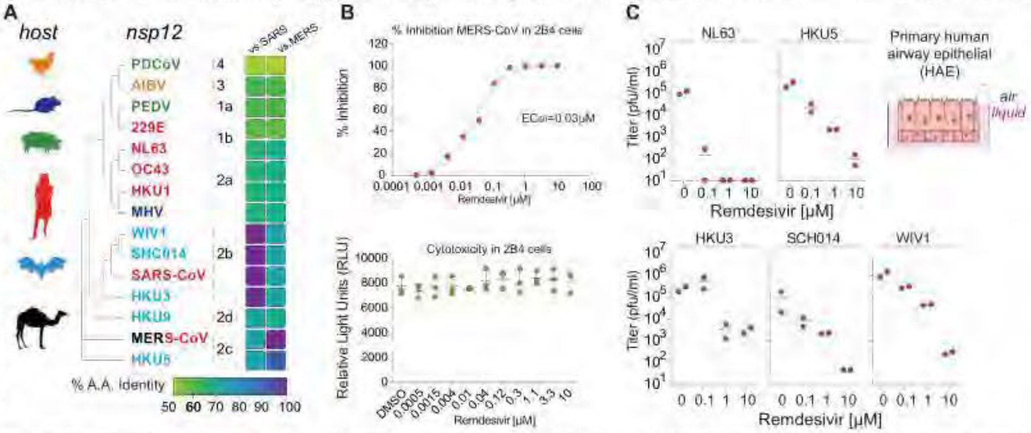

Figure 1. Combating viral genetic diversity with broad-spectrum antivirals. (A) RNA-dependent RNA polymerase (RdRp, nsp12) is conserved across the antigenically diverse CoV family. (B) Remdesivir exhibits a robust antiviral effect against MERS-CoV in human lung carcinoma 2B4 cells without toxicity. (C) Remdesivir is antiviral against a genetically diverse panel of human and bat CoVs in primary human airway epithelial (HAE) cultures, which model the human conducting airway.

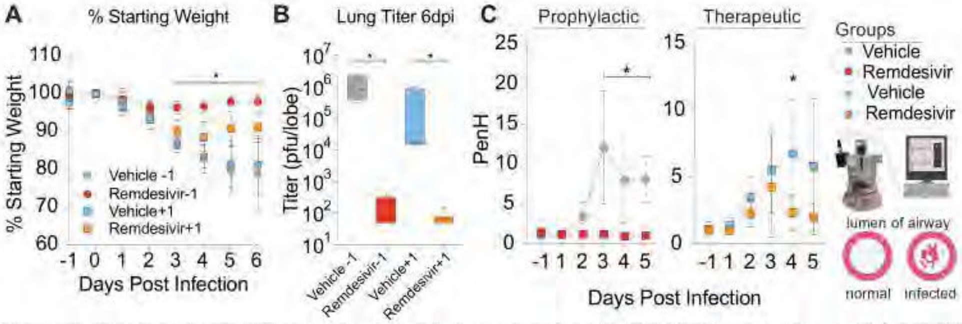

Figure 2. Prophylactic and therapeutic remdesivir ameliorates MERS-CoV disease. Groups of ten (50% male/female) 10-week old *Ces1c*$^{-/-}$ hDPP4 mice were treated with subcutaneous vehicle or 25mg/kg remdesivir BID beginning one day before (-1 dpi) or one day after (+1 dpi) infection. Mice were intranasally infected with 5E+05 PFU mouse adapted MERS-CoV (MERSp35C4). (A) Percent starting weight. (B). Virus lung titer at 6 dpi. (**C**) Whole body plethysmography to assess pulmonary function. PenH, a surrogate marker of airway obstruction, is shown. *, $P<0.05$ as determined by 2-way ANOVA or Mann-Whitney test.

Advances in CoV antiviral development. CoVs encode the largest known RNA virus genomes, 28-32 kb. Following cell entry, CoV genomic RNA is translated to yield 16 nonstructural proteins (nsps), of which nsp7-16 are likely to form a complex responsible for genome replication and subgenomic RNA synthesis[27,28]. The RNA-dependent RNA polymerase (nsp12-RdRp) is highly conserved across CoV genera (~70-90% amino acid identity) (**Fig. 1A**), making it an appealing drug target[29]. In addition, CoVs are unique among RNA viruses by encoding an RNA-dependent proofreading 3'-5' exoribonuclease (nsp14-ExoN) that is critical for high-fidelity viral replication and resistance to nucleoside analogues ribavirin (RBV) and 5-fluorouracil (5-FU)[30]. Thus, it is crucial to define polymerase inhibitors resistant to nsp14-ExoN-mediated proofreading.

During the current Centers for Excellence in Translational Research (CETR) project period supporting the Antiviral Drug Discovery and Development Center (AD^{3}C), we identified potent broad-spectrum anti-CoV nucleoside analogues, remdesivir (GS-5734; Gilead Sciences, **GS**) and EIDD-1931/2801 (Emory Institute for Drug Development, **EIDD**). Remdesivir is highly efficacious against MERS-CoV, SARS-CoV, and other zoonotic and endemic human CoVs[31] (**Fig. 1B,C**), thus demonstrating significant breadth in spectrum against multiple genetically and antigenically diverse CoVs (**Fig. 1A**). Additionally, prophylactic and therapeutic remdesivir inhibited SARS- and MERS-CoV disease in mice (**Fig. 2** and **3**)[31].

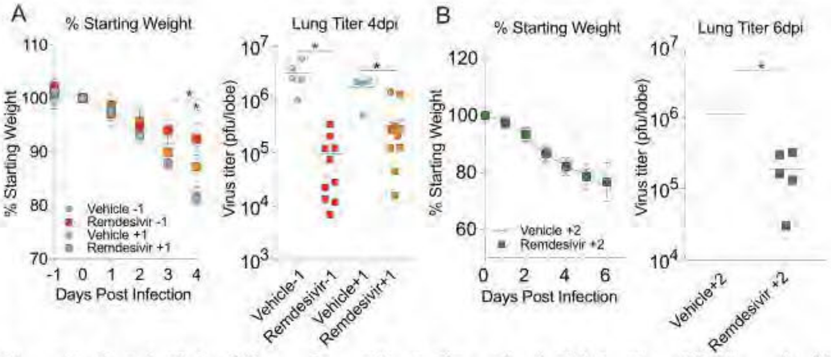

Figure 3. Remdesivir efficacy depends on time of administration. 20-25 week-old C57BL/6 *Ces1c*$^{-/-}$ mice were administered 25 mg/kg remdesivir or vehicle daily beginning on (A) -1 dpi or +1 dpi or (B) +2 dpi. Mice were infected with 1000 PFU mouse-adapted SARS MA15 and weighed daily. On 4 or 6 dpi, mice were euthanized, lungs resected, and viral load determined by plaque assay. *, $P<0.05$ as determined by Mann-Whitney test.

(b) (4)

(b) (4)

(b) (4)

[redacted] (b) (4) Hit-to-lead determination of analogues will be driven by structure-activity relationship (SAR) building assays by SR, UNC, and VUMC. If readily soluble and highly efficacious SR leads with broad CoV activity are identified, we will initiate preclinical development as described in this proposal for EIDD-1931/2801. If not, we will discontinue evaluation.

Combination treatment to enhance efficacy, limit resistance, and extend the therapeutic window. While the primary goal of this proposal is to accelerate the development EIDD-1931/2801, a complementary objective is to design and test combination therapies for additive or synergistic activity in order to increase spectrum and/or increase the barrier for resistance. Initially, we will combine remdesivir and EIDD-1931/2801, but SR leads and GS/EIDD backup compounds also will be considered. Additionally, we will use robust animal models to test whether potent antivirals combined with immunomodulatory agents can reduce both virus replication and pulmonary immune pathology. The scientific premise for this approach is two-fold. First, SARS- and MERS-CoV infection of mice and humans can result in severe acute respiratory distress syndrome (ARDS). Attempts to curtail immune pathology in patients with corticosteroids in the absence of effective antivirals did not improve disease outcomes and even delayed viral clearance in the upper respiratory tract[40,41]. Second, we have shown that while therapeutic remdesivir reduces virus replication, it does not protect mice from SARS-CoV disease if administered after the initiation of immunopathology (**Fig. 3**). Such an approach was successful in reducing autoimmune sequelae following chikungunya virus (CHIKV) infections[42]. We are exceptionally well positioned to determine the potential of combined potent DAAs and immunomodulators (see Aim 3) to extend the therapeutic window. Further, our studies will provide an evidence basis for combining commonly used immmunomodulatory drugs—steroids and IFN—with effective antivirals in patients with emerging viruses such as MERS-CoV toward potential clinical benefit.

B. INNOVATION. We have developed reverse genetics systems for multiple human, pre-epidemic, and zoonotic CoVs[43–45]. With these tools, we will determine the activity breadth and impact of passage-selected resistance mutations on replication, fitness, and pathogenesis across CoV genera. To understand drug uptake, metabolism, and antiviral effect in cells targeted by emerging CoVs, we will use primary HAE cultures, which recapitulate the cellular complexity and architecture of the human conducting airway[46–48]. The PK/PD of drug candidates will be assessed in our SARS-, MERS-, and bat-CoV mouse models of pathogenesis[31]. We will measure multiple clinically translatable metrics of CoV disease including virus lung titer, lung histopathology, respiratory function (whole body plethysmography), weight loss, blood cell counts (e.g. lymphopenia, neutrophilia), and inflammatory cytokines (**Fig. 5**)[31,49]. Next, we test combinations of CoV DAAs and host immunomodulatory agents for the potential to diminish virus replication and abrogate immunopathology using the methods above. Finally, we will integrate data from deep- and long-read next-generation sequencing (NGS) of CoV RNA from drug-treated cell lines, HAE cultures, and lung tissue from infected mice to determine the effects of antiviral compounds on viral RNA synthesis, fidelity, and lethal mutation accumulation to comprehensively define mechanism of action (MOA).

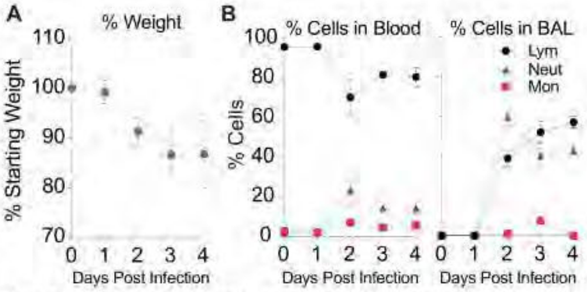

Figure 5. SARS-infected mice develop lymphopenia and neutrophilia. 10-week old C57BL/6 mice were infected with 1E+05 PFU of mouse-adapted SARS-MA15. (A) Percent starting weight. (B) Percent lymphocytes, neutrophils, and monocytes in blood and bronchoalveolar lavage (BAL).

C.1. SCIENTIFIC PREMISE. There are no approved therapies to effectively treat any human CoV infection. The scientific premise of this program is that small-molecule antivirals, singly and in combination, offer considerable promise for the control of current (MERS-CoV) and future emerging CoV epidemics. Additionally, we propose that broadly acting therapies can be developed that target more than one virus and more than one virus family to maximize their benefit to global public health. Data demonstrating the broad-spectrum antiviral efficacy and potential distinct activities of remdesivir (**Fig. 1-3**) and EIDD-1931/2801 (**Fig. 8** and **11**, **Table 1**) support this premise. The goal of our program is to generate comprehensive preclinical IND-enabling data for novel broad-spectrum anti-CoV monotherapies and to generate proof-of-concept data for various combination therapies.

C.2. EXPERIMENTAL RIGOR AND CONSIDERATION OF RELEVANT BIOLOGICAL VARIABLES. We are committed to conducting rigorous, unbiased and transparent research that includes multiple repeat experiments with replicates to ensure reproducible, statistically supported experimental outcomes. Cell lines, including primary cells, are routinely monitored for purity using histological analysis of cell morphology and by short terminal repeat marker profiling for extraneous contaminants (e.g., *Mycoplasma*). To reduce biological variability in cell-based *in vitro* antiviral analyses, we perform assays in multiple complementary cell types (e.g., continuous cell lines and primary cells) and using multiple complementary methods (e.g., infectious virus production and viral mRNA transcription). Our *in vivo* efficacy studies are carefully designed throughout this proposal, using reproducible established experimental designs and sufficient animal numbers including both sexes, different ages, and complementary endpoints. Thus, success or failure is not dependent on a single variable. To ensure the genetic identity of transgenic mice, we genotype using state-of-the-art arrays[50]. To ensure the genetic identity of recombinant viruses and wild-type (WT) viruses, we will perform whole-genome sequencing and/or PCR-based confirmation of unique marker mutations. To safeguard reproducibility across the scientific community, we have made available our virus strains, cells, and mouse models through BEI or MMRRC.

(b) (4)

[redacted]

D. TEAM COMPOSITION, EXPERTISE AND INTEGRATION.

(b) (4)

[redacted]

(b) (4)

[redacted]

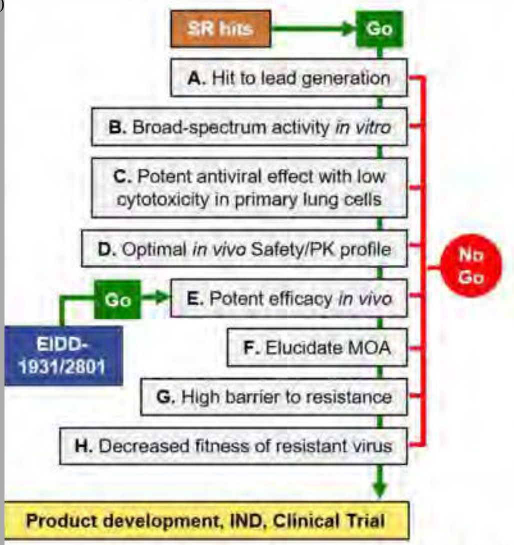
SR hits
Go
A. Hit to lead generation
B. Broad-spectrum activity *in vitro*
C. Potent antiviral effect with low cytotoxicity in primary lung cells
D. Optimal *in vivo* Safety/PK profile
No Go
Go
E. Potent efficacy *in vivo*
EIDD-1931/2801
F. Elucidate MOA
G. High barrier to resistance
H. Decreased fitness of resistant virus
Product development, IND, Clinical Trial

Figure 7. Go/No-Go decision tree for preclinical development of broad-spectrum antivirals for emerging CoV. This decision tree defines our criteria for the progression of lead preclinical development of towards IND. Since EIDD-1931/2801 is already a lead, it enters the tree midway through. A hit will become a lead if: (A) SARS-CoV EC_{50}/EC_{90} <0.25μM/<1μM; CC_{50} >30 μM; viral titer reduction (VTR) >3 logs @ 10 μM; MLM $t_{½}$ >60 min; (B) it is highly active against multiple group 2b CoVs; (C) it exerts similar antiviral effect in primary lung cells as in "A"; (D) it is well tolerated with suitable PK (bioavailability > 30%, $t_{1/2}$ >2 h, bioavailable in lung, etc.); (E) it is prophylactically and therapeutically efficacious *in vivo* against SARS- and/or MERS-CoV (<10% weight loss, <2 PenH score, >2 VTR 2 dpi); (F) its MOA is elucidated; (G) it has a high barrier to resistance (>10 passages); (H) viral resistance results in diminished fitness *in vitro/in vivo*.

Cross-Project Synergy across AD^{3}C. We will regularly share data in our monthly AD^{3}C CETR teleconferences, especially since EIDD-1931 demonstrates strong activity against CHIKV and VEEV (Project 2) and Zika virus (Project 3). We will communicate our data on MOA, drug resistance, and synergy of DAAs and immunomodulatory agents with

Projects 2 and 3 as data are acquired. Combination treatments built from EIDD-1931/2801, remdesivir, and any successful hit-to-lead CoV-active SR compounds not only carry the potential to improve breadth, potency, and resistance profiles against CoVs, but also might create bridge regimens effective against disparate virus families. Thus, the preclinical development program for CoV antivirals will promote progress across all AD^{3}C projects and potentially define novel, broad-spectrum antivirals.

Drug Development Pipeline. Four parallel product development pipelines exist within the program, including pre-IND data for: **i**) EIDD-1931/2801 and any lead compounds from SR, **ii**) any newly discovered broad-spectrum nucleoside analogues from Gilead and EIDD, **iii**) DAA combinations, and **iv**) combinations of DAAs and immunomodulators. In each pipeline, clear Go/No-Go criteria provide focus and programmatic direction (**Fig. 7**). Initially, we focus on generating comprehensive IND-enabling preclinical data packages for monotherapies (e.g., EIDD-1931/2801, SR leads, etc.). Second, we evaluate the potential for antiviral combinations to surpass the activities of monotherapy. Finally, we investigate the combination of DAAs and immunomodulators to cooperatively reduce both virus replication and excessive inflammation to more comprehensively treat disease and extend the therapeutic window. The overall goal for our program is to obtain pre-IND-enabling data for single antivirals, leverage data to obtain new funding for non-human primate efficacy studies, and generate proof-of-principle data on combination therapies that significantly improve outcomes.

E. APPROACH

E.1. SPECIFIC AIM 1. *To determine the spectrum of antiviral activity and therapeutic efficacy EIDD-1931/2801 and perform hit-to-lead stage evaluation of SR compounds.*

E.1.a. Rationale and overall experimental design. Our team has moved remdesivir towards IND and human clinical trial; however, there is still a need for continued discovery and development of additional anti-CoV therapeutics. Drug resistance may arise and/or new CoVs may emerge that are naturally resistant to monotherapies. The previous project period identified the nucleoside analogue/prodrug EIDD-1931/2801 as efficacious and highly potent against multiple β-CoVs of different groups including 2b (SARS-CoV), 2c (MERS-CoV) and 2a (MHV) in both continuous (Vero) and primary HAE cultures, with limited toxicity and with a putative MOA involving polymerase inhibition distinct from remdesivir. Thus, EIDD-1931/2801 will be the key focus of preclinical development in Aims 1 and 2 and combination treatment in Aim 3, although other leads and backups are also in development (below). In **Sections b-d**, we will assess the activity spectrum of EIDD-1931/2801 across divergent groups of CoVs; evaluate EIDD-1931/2801 metabolism, toxicity, and antiviral efficacy in HAE cultures; and determine the prophylactic and therapeutic efficacy of EIDD-1931/2801 and other leads against MERS- and SARS-CoV in murine models of disease. **Since EIDD-1931 and EIDD-2801* [redacted] (b) (4) *we will use the nomenclature, EIDD-1931/2801, throughout, with exception where experiments have already been performed with either compound or where indicated for specific experiments.* Concurrent with above experiments, in **Section f** we will perform "hit-to-lead" evaluation of SR-36097 and SR-35923. Chemical analogues of SR-36097 and SR-35293 will be subjected to a defined series of SAR assays at SR (Assay Core and Medicinal Chemistry and Lead Development Core), and VUMC and UNC will perform efficacy and toxicity studies to determine whether any compounds meet the criteria of a "lead" (**Fig. 7**). Other candidates in the pipeline that meet Go/No Go criteria also will follow the experimental route described below for EIDD-1931/2801.

E.1.b. EIDD-1931/2801 activity spectrum across CoV groups and genera. To define the activity of EIDD-1931/2801 and other nucleoside analogues across genetically diverse mammalian α- and β-CoVs, we will infect permissive continuous cell cultures with a low multiplicity of infection (MOI) of each CoV, incubate in the presence of a dose range of drug, and calculate EC_{50} values. We will use a MERS-CoV (β-2c CoV) reporter virus expressing nanoluciferase (MERS-nLuc) and monitor virus replication in Calu-3 human lung cells by quantifying luciferase. To assess family-wide spectrum, we will treat cells with drug and infect permissive cell types with the following viruses: α–1b HCoVs 229E and NL63; β-2a CoVs MHV and HCoV-OC43; β-2b SARS-CoV and related SARS-like bat CoVs HKU3-SRBD, WIV1 and SHC014; β–2c MERS-like bat CoV HKU5-S; β–2d bat CoV HKU9; and the β-porcine CoV, PDCoV. The effects of EIDD-1931/2801 on virus replication will be determined by plaque assay and qRT-PCR. Cytotoxicity (CC_{50}) will be assessed in similarly treated non-infected cultures by CellTiter-Glo assay. These experiments will define the CoV-inhibition breadth of EIDD-1931/2801 and establish the EC_{50}, CC_{50}, and selectivity index (EC_{50}:CC_{50}), all key data required for an IND application.

E.1.c. EIDD-1931/2801 antiviral efficacy, metabolism, and cytotoxicity in HAE. To determine the rate of EIDD-1931/2801 metabolism in the airway, we will measure [redacted] (b) (4) in HAE cultures in collaboration with EIDD. HAE will be treated with 1 μM and 0.1 μM EIDD-1931/2801, after which cultures will be harvested over time (e.g., 2, 8, 24, 36 hours (h) post-treatment) to quantify amounts of parental nucleoside and pharmacologically active TP by LC-MS at EIDD. To determine EC_{50} of EIDD-1931/2801 against MERS-CoV in HAE and related primary lung cells targeted by MERS-CoV (alveolar type II cells, primary lung fibroblasts, and primary lung microvascular endothelial cells[46,55]), cultures will be treated with a dose range of drug and infected with MERS-CoV. Virus titers in apical washes will be quantified by plaque assay. Toxicity will be determined by CellTiter-Glo and qRT-PCR for apoptosis-related gene expression (e.g., *Bax*, *Bad*, *Bcl-2*, *Fas*, etc.) as previously described[31]. These experiments will establish the relationship between levels of drug exposure and TP accumulation within cells, which will guide selection of dose for *in vivo* studies (**Section d)**.

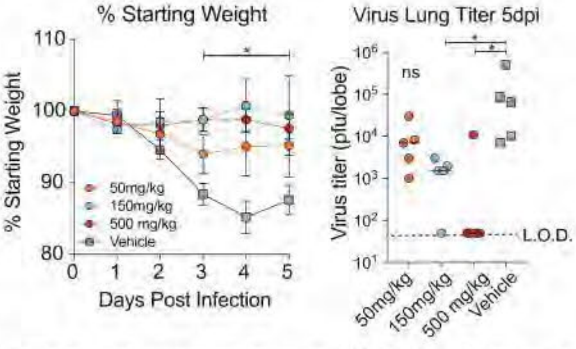

Figure 8. Oral EIDD-2801 prevents SARS-CoV pathogenesis. Groups of ten 20-week old female C57BL/6 mice were administered EIDD-2801 or vehicle beginning -2 h prior to infection and every 12 h thereafter (BID). Mice were intranasally inoculated with 1E+04 PFU mouse-adapted SARS MA15, after which body weight was measured daily. On 5 dpi, lung virus titer was determine by plaque assay. *, *P*<0.05 as determined by one-way ANOVA.

E.1.d. *In vivo* efficacy of EIDD-2801 in models of MERS and SARS pathogenesis. Using existing EIDD-1931/2801 mouse PK data (see EIDD PDP), we have initiated *in vivo* dose-finding studies with the mouse-adapted (MA) SARS-CoV MA15 strain (**Fig. 8**). 20-week old mice (N = 5/group) were treated orally with vehicle (10% PEG-400, 2.5% Cremophor RH40 in water) or 50, 150, or 500 mg/kg EIDD-2801 beginning -2 h prior to infection and every 12 h thereafter for 5 days. Mice were intranasally inoculated with 1x10^4 plaque-forming units (PFU) SARS-CoV MA15, body weight was measured daily, and mice were euthanized at 5 days post-infection (dpi). Our results suggest that treatment with 50 mg/kg EIDD-2801 provided partial protection from SARS-CoV MA15-induced weight loss. Mice treated with 150 or 500 mg/kg EIDD-2801 did not lose weight, and lung viral titers were significantly reduced. Treatment with 500 mg/kg also significantly diminished lung hemorrhage (data not shown). Based on these data, we propose to perform similar studies with MERS-CoV. Briefly, we will orally administer prophylactic vehicle or 50, 150, or 500 mg/kg EIDD-2801 (N = 12/group) beginning -2 h prior to infection of 20-25 week-old male (M) and female (F) C57BL/6 288/330 hDPP4 mice with 1x10^5 PFU of MERS-CoV P35C4 strain, a highly virulent MA isolate[58]. EIDD-2801 or vehicle will be administered every 12 h thereafter for 5 days. Mice will be monitored daily for clinical signs of infection including weight loss and impaired pulmonary function. At 5 dpi, mice will be euthanized, and lung hemorrhage evaluated by visual scoring, lung pathology assessed by histology, complete blood count (CBC) and blood cells in bronchoalveolar lavage (BAL) quantified by Vetscan HM5, inflammatory cytokines in serum and lung quantified by Bioplex, and viral loads determined by plaque assay[31]. Thus, we will comprehensively evaluate the antiviral effect of EIDD-2801 against MERS-CoV using multiple clinically translatable metrics. After dose finding, we will perform therapeutic efficacy studies with both SARS- and MERS-CoV. Briefly, we will compare four treatment groups: prophylactic vehicle or compound beginning -2 h prior to infection, and therapeutic vehicle or compound beginning +1 dpi (N = 12 mice/group). Infectious doses for each virus, compound dose route, vehicle composition, and metrics for efficacy will be similar to that above. If the compound proves therapeutically efficacious, we will perform similar studies, but extend the time of addition to begin treatment at 2, 3 or 4 dpi to determine the latest time in which treatment can be initiated and provide benefit. Similar studies also will be performed with SR-36097 and SR-35293 series leads or other GS/EIDD leads that satisfy **Go** criteria *in vitro*.

Drug efficacy in immunosuppressed models. MERS and SARS is most severe in aged individuals. Therefore, therapeutic efficacy of EIDD-2801 in younger animals will prompt testing in very stringent aged models. We have pioneered models of age-related disease exacerbation with emerging CoVs for evaluation of therapeutics[59,60]. Briefly, dose-finding and therapeutic efficacy studies for SARS- and MERS-CoV will be performed as above in 1.5 year-old male and female C57BL/6 (SARS-CoV) or C57BL/6 288/330 hDPP4 (MERS-CoV) mice. As lethal CoV pathogenesis in these aged models requires less virus as compared to young mice[61], the input dose for both SARS- and MERS-CoV will be reduced 1-2 log. CoV infections are known to cause severe life-threatening infections in immunocompromised humans[62]. Infection of mice lacking functional B- and T-cells (*Rag1*$^{-/-}$) with SARS- or MERS-CoV leads to the establishment of chronic lung infection without mortality (**Fig. 9**)[63,64]. To evaluate the capacity of EIDD-1931/2801 to clear virus in the absence of functional adaptive immunity, we will infect groups of 20 M and F C57BL/6 *Rag1*$^{-/-}$ (SARS-CoV) or C57BL/6 288/330 hDPP4 *Rag*1$^{-/-}$ (MERS-CoV) mice, which we recently generated (**Fig. 9**)[63]. Ten dpi, when chronic infection has been established, we will treat animals with an effective dose of EIDD-2801 (N = 20 (10M, 10F)) or vehicle (N = 20 (10M, 10F)) as described above. Clinical signs will be monitored daily as above. Groups of 5 mice will be

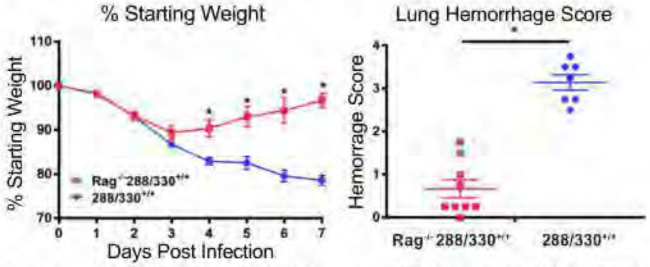

Figure 9. MERS-CoV pathogenesis is diminished in mice lacking adaptive immunity. Mice lacking functional B and T cells (*Rag*$^{-/-}$) were crossed with our humanized MERS receptor mouse line (288/300+/+, hDPP4) to generate a double transgenic line. 20-week old mice were intranasally infected with 2.5E+04 PFU mouse-adapted MERSP35C4. Percent starting weight and 7dpi lung hemorrhage score are shown. *, *P*<0.05 as determined by two-way ANOVA (weight) or Mann-Whitney test (hemorrhage).

euthanized at 15 and 25 dpi, and complete virological and clinical analyses will be performed as above. Additionally, lung tissue will be collected for viral and host RNA analysis in **Aim 2**. The goals of these studies are to evaluate drug efficacy in the absence of immune clearance mechanisms and potential for resistance emergence *in vivo*.

E.1.e. Feasibility, analysis and milestones. Our preliminary *in vitro* and *in vivo* studies with EIDD-1931/2801 support the likelihood of broad-spectrum anti-CoV activity and positive movement toward an IND submission. In addition, our successful and published studies with remdesivir[31] demonstrate our ability to execute the proposed studies with EIDD-1931/2801. The *in vitro* studies should be straightforward based on our experience and the capacity of EIDD-1931/2801 to penetrate continuous (Vero, Calu-3) and primary (HAE) cells and undergo metabolism to the pharmacologically active TP. Importantly, all of the human CoVs replicate efficiently in HAE cultures[16,31,65–67], the most biologically relevant experimental model for the human airway. Moreover, we have preliminary data demonstrating *in vivo* efficacy with oral EIDD-2801 against SARS-CoV (**Fig. 8**). We recognize that antiviral treatment of immunosuppressed animals is an extremely stringent test, and clearance may not be achieved. However, these studies will provide crucial insights into viral and host factors influencing treatment outcomes in individuals with defects in adaptive immunity, including potential for emergence of variants with reduced susceptibility. We note the availability of four additional nucleoside analogues with robust anti-CoV activity in cell culture, provided by EIDD and GS, which provide alternatives should EIDD-1931/2801 fail. Moreover, complementary experiments in **Aim 3** test the feasibility of combining DAAs with immunomodulators to extend the therapeutic window. If successful, we will pursue collaborations with Sina Bavari (USAMRIID, see letter of support) to evaluate drugs against SARS- or MERS-CoV in primates.

E.1.f. Hit-to-lead determination for SR compounds. Compounds SR-36097 and SR-35293 were identified as antiviral against SARS-CoV in a HTS at SR. We evaluated activity of these compounds against SARS- and MERS-CoV in HAE cultures (**Fig. 4**). Both SR-36097 and SR-35293 exhibited an antiviral effect against SARS-CoV without appreciable cytotoxicity. Neither compound inhibited MERS-CoV in HAE cultures. Thus, their potential as leads may be group 2b CoV-dependent, but are nevertheless worth pursuing through initial SAR hit-to-lead analysis based on their high activity against SARS-CoV. SR Assay Core and Medicinal Chemistry and Lead Development Core will triage hit-analogues via in-house SAR-building assays (i.e., SARS- and HKU3-SRBD-MA nanoluc and virus titer-reduction (VTR) assays). Details regarding the generation and optimization process can be found in the SR Medicinal Chemistry and Lead Development Core Research Strategy section. SR hits, along with new GS and EIDD hits, will be optimized based on antiviral activity, solubility, and microsomal (MLM) stability. Any confirmed optimized hits will be tested first for additional breadth of activity against group 2b SARS-CoV, SARS-like bat coronaviruses (HKU3/SHC014), group 2c MERS-CoV, and group 2a MHV at VUMC and UNC in continuous cell lines and HAE cultures (as appropriate). Any compound showing initial increased breadth, potency, and selectivity index will be considered for introduction into the development pipeline as above for EIDD-1931/2801 (**Fig. 7**).

E.2. SPECIFIC AIM 2: *To define MOA of EIDD-1931/2801 and impact of resistance mutations on* in vivo *therapeutic efficacy, viral fitness, and pathogenesis.*

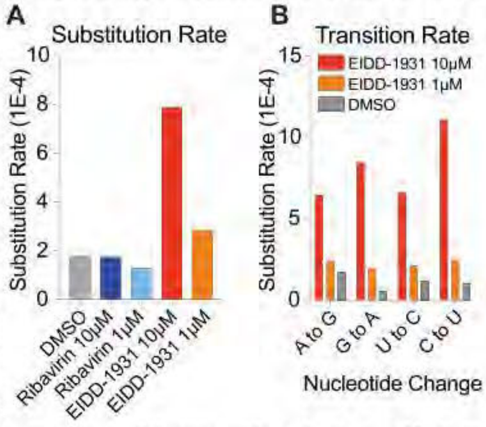

Figure 10. Treatment with EIDD-1931 leads to increased mutation frequency during MERS-CoV genome replication. Vero cells were pretreated with drug or vehicle for 24 h prior to infection with 0.01 PFU/cell MERS-nLuc. Medium containing drug or vehicle was added, and viral RNA was isolated from clarified supernatants at 48 hpi and prepared for Primer ID sequencing. (A) Substitution rate. (B) Transition rate.

E.2.a. Rationale. This Aim has two major goals. First, we will define the MOA of EIDD-1931/2801 using complementary virological and molecular methods. Second, through the passage selection and characterization of EIDD-1931/2801 reduced-susceptibility viruses, we will accumulate key data to support the progression of compounds to the clinical phase. Previously published data with VEEV[35] suggest that the principal mode of viral inhibition by EIDD-1931 is lethal mutagenesis through accumulated transition mutations in viral RNA. Our preliminary studies with MERS-CoV support a similar mechanism. Using Primer ID barcoding methodology developed in the Swanstrom laboratory at UNC[68,69], we found that EIDD-1931 treatment resulted in a four-fold increased mutation frequency (**Fig. 10**). The results also showed increased transition mutations consistent with EIDD-1931 incorporation, supporting the hypothesis that lethal mutagenesis may be one MOA. Experiments in this Aim will test this hypothesis rigorously as well as other likely MOAs for a nucleoside analogue, including premature chain termination and activation of innate immune responses by generation of viral pathogen-associated molecular patterns (PAMPS). In addition, passage of MHV and MERS-CoV in the presence of EIDD-1931 will be performed to select for resistance. This will catalyze several important steps toward drug IND: 1) define the genetic barrier to resistance; 2) identify putative resistance mutations and target viral proteins for antiviral MOA; 3) guide reverse-genetic testing across CoVs for conserved resistance determinants and mechanism; 4) enable testing of impact of resistance on virus replication, fitness *in vitro*, and pathogenesis *in vivo*; 5) establish effects of resistance on *in vivo* efficacy; and 6) determine impact of resistance on sensitivity to other nucleoside analogues. Our preliminary studies of passaged MERS-CoV and MHV in the presence of EIDD-1931 indicate that resistance may be only partial and difficult to achieve, but associated with mutations in the viral polymerase (nsp12-RdRp). The strategy and tools employed to better understand EIDD-1931/2801 MOA could be applied to SR-36097 and SR-35293 or chemical analogues if they pass hit-to-lead milestones. Structures of these compounds suggest they do not function as nucleoside analogues. Thus, passage for resistance and sequencing of any selected resistant isolates would be the first step in assessing MOA.

E.2.b. EIDD-1931/2801 MOA in continuous and primary cells *in vitro*. Our preliminary data support the hypothesis that one MOA of EIDD-1931 is incorporation mutagenesis (**Fig. 10**). However, drug treatment also may alter subgenomic RNA synthesis or generate truncated viral RNAs, which, when sensed by pattern recognition receptors of the innate immune system, could trigger potent antiviral responses and enhanced viral clearance. Direct antiviral action at the level of the polymerase or mutagenesis is testable in continuous cell lines (e.g. Vero, Calu-3). For both direct effects and innate immune response, HAE cultures are suitable to test these possibilities because they possess intact innate immune sensing and effector pathways [56,70].

RNA inhibition and mutagenesis. To define the direct and indirect antiviral effects exerted by EIDD-1931/2801, we will infect continuous cell lines and primary HAE cultures with SARS-CoV, SARS-CoV-ExoN(-) (no proofreading), and MERS-CoV with a range of EIDD-1931/2801 concentrations. At 12, 24, and 48 h post-infection (hpi), progeny virions will be harvested from culture supernatants or HAE apical washes, and total RNA will be purified from each. We will use Primer ID to define mutation frequency and types for amplicons across the genome. In parallel, we will perform long-read single-molecule sequencing using the Nanopore MinION system on both total infected-cell and packaged viral genomic RNA to determine if treatment causes an increase in premature chain termination and/or packaged defective genomes. Nanopore technology for direct RNA sequencing is now established at both VUMC and UNC. Finally, we will examine the effect of treatment on virion specific infectivity by comparing ratios of viral genomic RNA to particle infectivity. It is possible that EIDD-1931/2801 may have alternative direct effects, such as inhibiting nsp12-RdRp or interfering with nsp14-ExoN mediated proofreading. We would pursue these MOAs if, for example, we observed mutagenic changes that did not correlate with direct incorporation of EIDD-1931/2801.

Induction of antiviral immunity. Using HAE, Calu-3, and Vero cells to determine if drug treatment triggers an enhanced innate antiviral response, we will perform qRT-PCR with primer/probe sets specific to interferon (IFN)-stimulated genes (ISGs) and other innate immune effector genes on NGS libraries prepared from total RNA collected above[53]. qRT-PCR also will be used determine the ratio of viral genomic RNA to subgenomic RNA. Nanopore MinION long-read single-molecule sequencing will be performed on total RNA to determine if viral genomic and subgenomic RNA length is altered by treatment. If drug treatment leads to generation of altered or an increased abundance of viral RNAs, or inhibits expression of virally encoded IFN antagonists, we would expect to see significantly elevated levels of ISG transcripts and increased inhibition of virus replication in HAE cultures but not in Vero cells, as the latter cell type does not produce interferon in response to viral infection. For example, host RNA-editing enzymes and ISGs APOBEC3G, APOBEC3A, and ADAR, may not be expressed in Vero cells, but may contribute to a lethal mutagenesis of EIDD in IFN-competent cells, especially in the absence of ExoN function[70–72]. Additionally, NGS analysis similar to those above on lung tissue isolated from $\mathit{Rag}^{-/-}$ mice (**E.1.d**) chronically infected with SARS, SARS ExoN(-), or MERS-CoV and treated with EIDD-2801 or vehicle will reveal specifically whether innate immunity is differentially induced upon treatment. If collective results of sequence/expression analysis suggest that specific host pathways or molecules, for example host 3'-5' exonucleases, are driving a robust innate immune response toward a cumulative antiviral effect, the contribution of host molecules will be confirmed using siRNA knock-down or CRISPR-Cas9 gene-editing (knock-out) approaches in Calu-3 cells[53].

E.2.c. Selection for EIDD-1931/2801 resistance. We have passaged MERS-CoV and MHV in the presence of increasing concentrations of EIDD-1931 and identified mutations in MHV and MERS-CoV nsp12-RdRp and in the MHV nsp14 exoribonuclease-enhancing cofactor, nsp10 (**Table 1**), suggesting that multifactorial drug resistance mechanisms may occur, as we have recently shown for remdesivir[51]. We will continue passage of MERS-CoV and MHV in continuous cell lines (Vero) and initiate passage of MERS-CoV in Calu-3 cells and HAE cultures in the presence of stepwise increasing concentrations of EIDD-1931/2801, as we previously reported for remdesivir[51]. Passage will be performed in multiple parallel

Virus-strain	Exp.	Pass.	[EIDD-1931] (Fold IC_{50})	nsp12-RdRp Mutations	nsp10 Mutations
MHV-A59	1	17	4 μM (~25x)	V174M, A234T, T552I, H709Y	V73I
	2	8	8 μM (~50x)	M288I, K474R, N548S	n.d.
MERS-EMC/2012	1	16	4 μM (≥5x)	V558I	n.d.

Table 1. Identification of EIDD-1931 resistance mutations in MHV and MERS-CoV. MHV-A59 and MERS-EMC/2012 were serially passaged in medium containing EIDD-1931 in stepwise increasing concentrations. At passage 17 and 8 (MHV) and 16 (MERS-CoV), total infected-cell RNA was collected, and nsp10 and/or nsp12 gene sequence was determined. Exp.: experiment. n.d.: not determined.

(concurrent) lineages to allow for selection for alternative pathways to resistance. Passaged virus displaying phenotypic resistance, including replication and cytopathic effect (CPE) at higher drug concentrations and decreasing MOI, will be tested in EC_{50} and log-reduction assays to confirm and quantify the resistance phenotype. Using NGS of terminal-passage virus RNA from culture supernatant and total RNA from infected-cell cultures, we will identify a consensus population and minority variants across the full-length CoV genome[73]. Putative resistance mutations will be introduced into the parental MERS-CoV infectious clone individually and in combination with other identified mutations using reverse genetics[43], and EC_{50} values of recovered virus will be compared with passaged-virus populations. Priority will be given to mutations in nsp12-RdRp, nsp10, and nsp14-ExoN since these proteins are critical for RNA synthesis and replication fidelity. Confirmed resistance mutations will be aligned with SARS-CoV and other disparate CoVs to determine if resistance determinants are conserved across the CoV family. Modeling of mutations onto solved (nsp14, nsp10) or modeled (nsp12) structures will reveal potential functional consequences and further inform the selection of mutations to reintroduce and test.

E.2.d. Impact of resistance mutations on MERS- and SARS-CoV replication, fitness, and virulence.

Replication. To define the impact of drug resistance mutations on viral replication, we will perform single-cycle (high MOI) and multi-cycle (low MOI) infection assays in Calu-3 cells using plaque-cloned resistant viruses and those with specific engineered putative resistance mutations, in parallel with WT MERS- or SARS-CoV. RNA from supernatant and cells will be isolated, and viral loads in supernatants quantified by plaque assay. Genomic and subgenomic RNA levels will be quantified by RT-qPCR at times corresponding to peak viral RNA synthesis. We also will assess the impact of resistance mutations on replication fidelity and RNA length using Primer ID and MinION NGS methods, respectively. Replication fidelity is a known CoV virulence determinant[63]. If replicative, fidelity, and fitness (see below) phenotypes are altered, we will confirm these findings in primary HAE cultures.

Competitive fitness. The comparative fitness of resistance mutants and WT virus to EIDD-1931/2801 will be determined through co-infection/passage experiments. Vero cells will be co-infected at WT:mutant ratios of 1:1, 9:1, and 1:9, followed by five sequential supernatant passages, after which culture medium (progeny virions) and total RNA (viral genomes and viral mRNA) will be collected. WT ("barcoded" by neutral synonymous changes) and mutant virus will be simultaneously quantified by multiplex RT-qPCR [63].

Virulence. Mutant viruses demonstrating replicative fitness equal to or greater than WT virus will be further tested in HAE cultures as described above and prioritized for *in vivo* experiments to evaluate the impact of resistance mutations on CoV replication and virulence in murine models of SARS (20-25 week-old C57BL/6 mice) and MERS (20-25 week-old C57BL/6 288/330 hDPP4 mice) pathogenesis. Mice will be inoculated intranasally with high ($1x10^5$ PFU), medium ($1x10^4$ PFU), and low ($1x10^3$ PFU) doses of WT SARS-CoV MA15, WT MERS-CoV P35C4, or isogenic viruses encoding resistance mutations (N = 12/group; 6F/6M) and monitored daily for clinical signs of disease, including weight loss and impaired pulmonary function. Mice will be euthanized at 7 dpi, or earlier if maximum allowable weight loss has been achieved, and virological and clinical analyses will be performed as in **E.1.d**. By applying a range of doses from high, which requires fewer rounds of replication to reach peak titers in the lungs, to low, which requires more rounds of replication to reach peak titers in the lungs, we will capture disease signs ranging from lethality to transient weight loss to lack of observable disease. These experiments will enable evaluation of the pathogenic impact of resistance *in vivo*[51].

Analysis and milestones. Based on our experience with remdesivir and preliminary data with EIDD-1931, we expect these experiments to identify the range of possible resistance pathways and to define the speed or difficulty of selection for resistance. While resistance mutations could exert positive, neutral, or negative effects on viral replication, fitness, and virulence, to date all selected and engineered mutations (not involving EIDD-1931) in the MHV and SARS-CoV nsp12-RdRp have resulted in loss of virus fitness *in vitro* and attenuation *in vivo*[29,31,51]. Our previous studies with the CoV resistant to the nucleoside analogue remdesivir demonstrate trans-clade diminished replicative fitness and virulence. Based on the slow emergence of EIDD-1931 resistance and low tolerance of virus to increased EIDD-1931 concentration, we hypothesize that EIDD-1931/2801 resistance will be partial and result in decreased competitive fitness *in vitro* and virulence *in vivo*. Analysis of multiple lineages and an ability to test across CoVs by recombinant engineering will allow a comprehensive understanding of possible resistance pathways and their implications for viral pathogenesis, all critical considerations for use of EIDD-1931/2801 in humans. If host RNA-editing enzymes (AOPBEC3G/A, ADAR, etc.) appear to contribute to lethal mutagenesis, cell lines ectopically over-expressing these enzymes or CRISPR/Cas9-engineered null cell lines will be generated to investigate their potential contributions.

E.2.e. Impact of resistance mutations on EIDD-2801 therapeutic efficacy *in vivo*. To test this, 20-25 week-old C57BL/6 (SARS-CoV) or C57BL/6 hDPP4 (MERS-CoV) mice will be inoculated intranasally with a high dose of SARS-CoV MA15, resistant SARS-CoV MA15, MERS-CoV P35C4, or resistant MERS-CoV P35C4 (N = 10 (5F, 5M) mice/group) and monitored daily for clinical signs of infection, including weight loss and pulmonary function. We will initiate drug treatment at 12-24 hpi depending on the efficacious dose and dosing frequency established in **Aim 1**. Clinical disease will be measured as in **E.1.d**. At 5 dpi, mice will be euthanized, and virological and clinical analyses will be performed as in **E.1.d** to determine antiviral efficacy. We expect viruses that display resistance to drug treatment *in vitro* (**Section c**) to be similarly resistant to therapeutic treatment *in vivo*. The dose of input virus may change from what is proposed depending on the outcome of pathogenesis studies with recombinant resistant virus (see **E.2.d**). If resistant viruses are attenuated for pathogenesis at all doses tested, we still will explore effects of drug treatment on replication of resistant viruses.

E.2.f. Impact of resistance mutations on sensitivity to other drugs. We have preliminary results indicating that remdesivir resistance mutations in the MHV nsp12-RdRp (F476 and V553L) actually ***increase virus sensitivity to EIDD-1931***, a structurally distinct cytidine analogue (**Fig. 11**). We will determine whether EIDD-1931/2801 resistance mutations alter sensitivity to remdesivir and other nucleoside analogues or mutagens (e.g., ribavirin, 5-FU). WT and recombinant EIDD-1931/2801 resistance mutants of SARS- and MERS-CoV will be compared in antiviral dose-response assays in Calu-3 and Vero cells. In addition to remdesivir, we will test mutagens 5-FU, RBV and 5-azacytindine (5-AC) as well as the non-obligate chain terminator, 2' C-methyl adenosine (2'CMA), and SR-36097 and SR-35293 parent and/or prioritized derivatives that pass Go/No-Go criteria. Cross-resistance between EIDD-1931/2801 and all classes of nucleosides would suggest a general fidelity increase, whereas retention of sensitivity to one or more compounds would be consistent with nucleotide selectivity as the mechanism of EIDD-1931/2801 resistance and further support testing of combined DAA nucleoside analogues, specifically remdesivir and EIDD-1931/2801, as proposed in Aim 3.

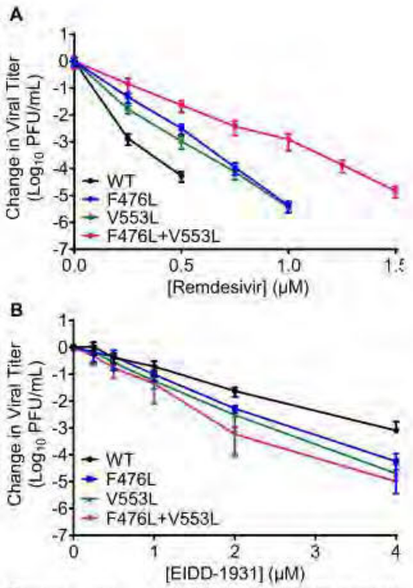

Figure 11. Impact of remdesivir resistance mutations on sensitivity to other drugs. Resistance mutations F476L and V533L (A) decrease sensitivity to remdesivir, but (B) increase sensitivity to EIDD-1931.

Feasibility, analysis, and milestones. Both our experience with remdesivir and our preliminary data on EIDD-1931 demonstrate team expertise and feasibility of proposed preclinical development strategies for EIDD-1931/2801. Innovation in this Aim includes application of Primer ID and MinION NGS to understand the evolutionary path and rate of drug resistance in the *Coronaviridae*. As CoVs encode a three-component fidelity repair system (i.e., nsp10, nsp12, and nsp14), it is possible that emergent resistance mutations will have profound impact on replication, transcription, and fidelity, revealing novel epistatic and additive interaction networks that regulate the relationship between fidelity and virulence. Although our early studies support the development of EIDD-1931/2801, we will work with investigators at EIDD and GS according to their product development plan and in coordination with AD^{3}C scientific advisors and executive committee to review all results and proceed through Go/No-Go decision points. For example, if studies demonstrate lack of *in vivo* efficacy for prophylaxis or early treatment, we would determine if alternate models or routes of administration are needed. Similarly, if we determine that rapid resistance occurs by multiple pathways, or that resistant viruses have increased virulence or altered disease, we will consider a No-Go decision.

E.3. SPECIFIC AIM 3. *To test combination regimens for treatment of emerging CoV disease.*

E.3.a. Rationale: The goal of Aims 1 and 2 is to generate an IND-enabling preclinical data package and progress EIDD-1931/2801, SR-leads, and other EIDD and GS leads toward human clinical trials. With **Aim 3**, we build upon **Aims 1 and 2** and our previous data with remdesivir to generate proof-of-principle data that may expand and improve the utility of our monotherapies. By combining DAAs with each other or with immunomodulators, we will assess potential for additivity or synergy and increased breadth of activity, limiting or preventing resistance emergence, and extension of the therapeutic window for intervention. **Combining nucleoside DAAs.** Combinations of nucleoside analogues have been used successfully to treat multiple virus infections, including HIV, HCV, and influenza for synergy, prevention of resistance, and even cure[74–77]. Remdesivir is a phosphoramidate prodrug of the adenosine nucleoside analogue, GS-441524, and is proposed to function as a non-obligate chain terminator[78]. EIDD-1931/2801 is a cytidine analogue that our preliminary data suggest may function as an incorporation mutagen. To date, resistance mutations selected by the two nucleosides have been distinct and functionally opposed; resistance to remdesivir results in increased sensitivity to EIDD-1931 (**Fig. 10**). Thus, we will test this combination and others in collaboration with GS and EIDD. **DAAs and immunomodulators.** MERS- and SARS-CoV disease either resolves or results in mortality in approximately four weeks. While viral replication peaks 7-10 days after onset of symptoms, progression to severe disease is primarily driven by immunopathology[14,79], an observation that is corroborated in MA SARS-CoV-infected *Rag*$^{-/-}$ mice, which do not display disease symptoms despite lack of viral clearance.[63] Attempts to treat SARS and MERS patients with immunomodulators in the absence of effective antiviral therapy did not improve clinical outcomes and delayed viral clearance[80]. In this Aim, we will test whether concurrent targeting of virus replication and inflammation with combinations of potent DAAs and immunomodulators will diminish virus replication and immunopathology, thereby extending the therapeutic window and utility CoV DAAs. The potential impact of this Aim is extraordinarily high as it directly addresses the real-life situation that emerged in many SARS and MERS patients and can be extended to other severe acute respiratory virus infections like influenza.

E.3.b. Direct acting antivirals: EIDD-1931/2801 and remdesivir.

Efficacy, potency, and synergy. We will perform viral replication assays using combinations of remdesivir and EIDD-1931/2801 in a two-way dose matrix. Calu-3 cells will be infected with 0.01 PFU/cell nanoluciferase (nLuc)-expressing SARS- or MERS-CoV reporter viruses in the presence of drug combinations and each drug alone. Virus replication will be measured by nLuc assay at 48 hpi. Cytotoxicity will be assessed by biochemical assay (CellTiter-Glo) and microscopy in parallel uninfected drug-treated cultures. Results will be analyzed using established algorithms such as median-effects method, isobologram analysis, and synergy landscape plots[81] and compared to determine if drug interactions are antagonistic, neutral, additive, or synergistic.

Effect on RNA, mutation frequency, and resistance. If we identify additive, neutral, or synergistic efficacy/potency, we will perform parallel passage of virus with single and combined treatments to test for impact on viral RNA, mutation frequency, and resistance emergence. We will infect Calu-3 cells with SARS- or MERS-CoV in the presence of combination regimens at a starting concentration of 1-2x EC_{50} of each. Analysis of passaged virus will be as for EIDD-1931/2801 alone in Aim 1 and will include deep sequencing of viral RNA from infected cells and virions for mutation frequency and RNA truncations; Sanger sequencing of multiple plaque isolates of phenotypically resistant virus to identify linkage of candidate mutations; testing of plaque-isolate viruses for sensitivity to remdesivir and EIDD-1931/2801 alone and together; and reverse-genetic engineering of mutations from clonal and population virus to test individual and combinatorial contributions to resistance. Other DAA combinations can be evaluated as other drugs progress successfully through the pipeline.

DAA formulation and product characterization (formulation and toxicity in mice and cells). The PK, safety profiles, and dose regimens for EIDD-1931/2801 and remdesivir are established, but combination profiles will be created in collaboration with EIDD and GS. Prior to *in vivo* efficacy testing of combination EIDD-1931/2801 and remdesivir, we will first determine if PK/safety profiles are altered by co-administration. In collaboration with GS and EIDD, we will perform PK studies using combinations of previously determined *in vivo* effective doses of EIDD-1931/2801 (oral 150 or 500 mg/kg BID) and remdesivir (subcutaneous 25 mg/kg BID). If *in vitro* data demonstrate a strong additive or synergistic antiviral effect, the combination formulation may change.

Efficacy of combined DAAs in murine models of MERS and SARS disease. If we identify additive, neutral, or synergistic efficacy/potency *in vitro*, combinations have been deemed safe *in vivo*, and PK profiles of each component are not altered in combination, we will test *in vivo* therapeutic efficacy of combined EIDD-1931/2801 and remdesivir. Using established protocols, all experiments using remdesivir will be performed in mice deficient in carboxylesterase 1c (*Ces1c*) (C57BL/6 $Ces1c^{-/-}$ mice) as remdesivir has a relatively poor plasma stability in WT C57BL/6 mice due to expression of *Ces1c*, which is absent in humans[82]. $Ces1c^{-/-}$ mice also may prove useful for testing of nucleoside analogues in other AD^{3}C components including **Project 3**. We also have generated $Ces1c^{-/-}$ mice that support MERS-CoV infection (C57BL/6 288/330 hDPP4 $Ces1c^{-/-}$) (**Fig. 2**). Once the efficacious dose of the combination has been determined through prophylactic dose-finding studies similar to those in **E.1.d**, we will perform focused therapeutic efficacy studies consisting of four treatment groups (N = 10 (5F, 5M) mice/group,): vehicle, remdesivir, EIDD-1931/2801, and a combination of EIDD-1931/2801 and remdesivir. Mice will be inoculated intranasally with a high dose of either SARS-CoV MA15 or MERS-CoV P35C4 and monitored daily for morbidity (weight loss) and respiratory function via whole body plethysmography. At 5 dpi, animals will be euthanized, and virological and clinical endpoints will be evaluated as in **E.1.d**. Reduced weight loss and pathogenesis, improved lung function, and/or reduced viral loads as compared to each compound alone would suggest *in vivo* drug additivity. If we observe improved or neutral outcomes compared to individual compounds, we will 1) compare virus from lung homogenates treated with both drugs and each drug alone for evidence of resistance; 2) test whether *in vivo* combination treatment is effective against viruses resistant to EIDD-1931/2801, remdesivir, or both; and 3) evaluate combination treatment using immunosuppressed, 1.5 year-old aged, or $Rag1^{-/-}$ mice as described above. Combined, these experiments will provide a comprehensive understanding of the potential clinical utility for combining these nucleoside analogues.

E.3.c. Co-administered EIDD-1931/2801 or remdesivir and immunomodulators in MERS- and SARS-CoV-infected mice. Emerging CoV infections cause acute lung damage by direct viral injury to respiratory tissues and immune-mediated pathology[83]. SARS-CoV titers peak at 2 dpi in mice. Treatment with remdesivir after peak titer significantly reduced virus titers but did not improve clinical outcomes of SARS-CoV-infected mice (**Fig. 3**), indicating that immunopathology was initiated prior to treatment and disease could not be resolved by DAA alone. These data establish robust models for evaluating the impact of immunomodulatory/DAA combination therapies on disease outcomes. We propose to test whether co-administration of DAAs and immunomodulators (**Fig. 12**) will extend the

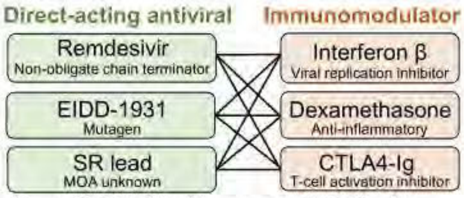

Figure 12. Combinations proposed in this Aim.

therapeutic window and possibly prevent severe disease by reducing virus titer and halting lethal progression of immunopathology. To test this, we will first co-administer remdesivir with corticosteroids or IFN-β. Both of these immunomodulators have been used in SARS and MERS patients[41], providing a clinical reference point for experimental design and interpretation. In addition to its antiviral activity, IFN treatment also induces ISGs that regulate inflammation, resolution, and repair, as well as RNA-editing enzymes like APOBEC3C/A or ADAR that could contribute to lethal mutagenesis[70–72,84]. 20-25 week old C57BL/6 *Ces1c*$^{-/-}$ mice will be inoculated with a 1x10^4 PFU dose of SARS-MA15. First, treatment will be initiated at 1 dpi, when remdesivir is known to be effective alone, although the animals experience some weight loss and virus titers are reduced, but not eliminated. Infected mice will be divided into four groups (N = 10 (5F, 5M)/group): vehicle, immunomodulator, remdesivir, and remdesivir/immunomodulator. Remdesivir (25 mg/kg) will be subcutaneously administered twice daily. The corticosteroid, dexamethasone, will be administered intraperitoneally once daily at a dose (10 mg/kg) that effectively mitigates influenza pathogenesis[85]. A human equivalent dose of IFN-β (1.6 million units; R&D Systems) will be administered subcutaneously every other day. The efficacy of dexamethasone and IFN-β will be monitored via CBC and number of inflammatory cells in bronchoalveolar lavage fluid (see **Fig. 5**). We will monitor virological and clinical endpoints as in **E.1.d**. Based on these results, in subsequent experiments, we will determine if the treatment window can be extended by initiating combination therapy on 2, 3, 4, 5, or 6 dpi. If immune suppression does not improve or worsens outcomes, we will systematically change the modulator dose, time of addition, or frequency to better understand the nature of the failure. As a backup, we will explore combinations of antivirals and immunomodulatory antibodies, such as 300 µg CTLA4-Ig, which is an approach shown to be effective at diminishing CHIKV titers and resultant immunopathology[42]. We will perform studies similar to those above using EIDD-1931/2801 or remdesivir in combination with CTLA4-Ig. Alternatively, we will couple a biologic antiviral (human monoclonal antibody, S227.14), which broadly neutralizes SARS-like viruses, with CTLA4-Ig[17,86]. Given our robust set of clinical measurements (e.g., death, respiratory function, weight loss), we are confident in our ability to sensitively detect changes in disease outcomes per condition.

E.3.d. Interpretation and milestones. This is a high-impact, ambitious Aim designed to address some of the most important and challenging questions in antiviral management of acute lung infections. We believe that these studies could pioneer new approaches for combating the immunopathologic component of severe respiratory infection, assisting in the development of new therapies for RSV, influenza, and other high-consequence respiratory viruses. EIDD-1931/2801 and remdesivir are both broad-based inhibitors against many emerging viral infections, and we note that combining these two nucleoside analogues offers considerable opportunity for not only more effective CoV therapies, but also a robust treatment modality off-the-shelf for rapid response in new CoV, filoviruses, paramyxovirus, and Lassa fever virus outbreak settings[31–35]. Therefore, we have carefully designed these experiments to yield clear and interpretable data for determination of proof-of-principle and potential for further development. If the combination of immunomodulators and effective DAAs blocks development of immunopathology while allowing effective reduction in virus titer and direct cell damage, resulting in improved clinical outcomes as compared to each monotherapy, more detailed studies of the impact on local vs. systemic virus replication and recovery will be designed, as well as studies to determine the point at which DAA or combined treatment no longer provides a clinical benefit. Alternatively, it is possible that early use of immunomodulators, even with effective DAAs, could be detrimental and allow for exuberant viral replication and direct tissue damage. While not desired, this would be an important outcome and provide guidance for any planned trials of DAAs in human CoV disease.

Project 1 Milestones	**Year 1**		**Year 2**		**Year 3**		**Year 4**		**Year 5**	
Quarter	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**
SPECIFIC AIM 1										
Activity spectrum across CoV groups	■	■	■	■	■					
Antiviral efficacy and cytotoxicity *in vitro*	■	■	■	■	■					
Efficacy against SARS- and MERS-CoV *in vivo*		■	■	■	■	■	■	■		
Hit to lead determination SR compounds	■	■	■	■	■	■	■	■	■	
SPECIFIC AIM 2										
Mechanism of action *in vitro*	■	■	■	■	■	■	■	■	■	■
Identification of resistance mutations	■	■	■	■	■	■	■	■	■	■
Impact of resistance mutations on viral fitness and virulence				■	■	■	■	■	■	■
Impact of resistance mutations on therapeutic efficacy						■	■	■	■	■
Impact of resistance mutations on sensitivity to other drugs							■	■	■	■
SPECIFIC AIM 3										
Combined efficacy and potency of DAAs *in vitro*	■	■	■	■	■	■	■	■	■	■
Combined efficacy of DAAs *in vivo*				■	■	■	■	■	■	■
Combined efficacy of DAA and immunomodulators *in vivo*		■	■	■	■	■	■	■	■	■

Table 2. Project 1 milestones and timeline.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes	● No
Is the Project Exempt from Federal regulations?	○ Yes	○ No
Exemption Number	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8	
Does the proposed research involve human specimens and/or data	○ Yes	● No
Other Requested information		

Vertebrate Animals

The goal of these studies is to accelerate the preclinical development of antivirals and combination therapies targeting emerging CoV infections. Animal research plays a key role in the development of medicine by providing evidence of therapeutic efficacy and insight into the pharmacokinetics, metabolism, safety and potential adverse events. This information is essential for the progression to human clinical trial. All rodent animal experiments will be performed at the (b) (3) (A), (b) (6) in dedicated facilities under the direction of the research PI. Prior to infection studies, the animals will be maintained in Sealsafe$^{\text{TM}}$ HEPA-filtered air in/out unit or compatible system for at least one week prior to virus challenge. In addition, our laboratory personnel inspect animals daily and any animal in distress is immediately euthanized (moribund, unresponsive, loss of more than approved percentage of starting weight). Animal care and housing at (b) (3) (A), (b) (6) recommendations and all personnel have attended mandatory (b) (3) (A), (b) (6) training courses. A trained veterinarian is always on call to assist with problems in animal care and husbandry. Below we summarize the description of procedures for each specific Aim, justifications, minimization and pain and distress and methods for euthanasia.

1. Procedures

All rodent work will be done at (b) (3) (A), (b) (6) in accordance with the Guide for the Care and Use of Laboratory Animals. The minimum numbers of animals will be used in order to achieve our experimental goals with statistical significance. We aim to monitor virus replication in live animals over time using in vivo imaging rather than traditional means of sacrificing multiple cohorts to gain similar data. In vivo imaging studies require fewer animals in step with the 3R principle (replacement, reduction and refinement).

E.1. Specific Aim 1: To determine the spectrum of antiviral activity and therapeutic efficacy of lead CoV-active compounds.

E.1.d. Specific Aim 1 part d. Evaluate top leads in murine models of SARS and MERS disease. If we are successful in developing promising leads in Aim 1 that have suitable pharmacokinetic (PK) profiles, we will perform in vivo efficacy studies with SARS- and MERS-CoV. All studies will be performed in 20-25 week old mice using equal numbers of males and females. We will first perform prophylactic dose finding studies followed by prophylactic and therapeutic efficacy studies.

Aim 1.d.A. In vivo dose finding for lead molecules in SARS-CoV efficacy studies

Rationale: PK and toxicity studies will determine optimal formulation and maximal tolerated dose (MTD) for a given route. Once this has been established, we will determine the optimal dose of lead that provides a robust antiviral effect through dose finding studies.

Drug Dosing: The formulation, route and frequency will be determined in Aim 1.2. We have experience with oral, subcutaneous and intraperitoneal dosing. Drug will be administered prior to infection. To repeat pilot studies with EIDD-2801, we will administer vehicle (10% PEG-400, 2.5% Cremophor RH40 in water) or 50, 150 or 500 mg/kg EIDD-2801 BID by the oral route.

Mice: Equivalent numbers of male and female 20-25 week old C57BL/6J (b) (3) (A), (b) (6) will be used for these studies. Each study will have four infected and treated groups: High, medium and low compound, and a vehicle control. Each treatment group infected with SARS-CoV will be comprised of 12 mice (6 male/6 female). To control for potential effects associated with dosing on animal weight or pulmonary function, a smaller “sham” infected group will also be included (n = 6).

Virus: A dose of 1E+04 plaque forming units (PFU) SARS-CoV MA15 strain (mouse adapted) will be delivered intranasally in 0.05ml. Virus will be diluted in phosphate buffered saline (PBS). Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi (days post infection), animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via bronchialveolar lavage (BAL).

Repetition: Since this study is simply intended to provide a prophylactic dose exhibiting an antiviral effect for future studies, upon repeating Experiment 1, not all doses may be required. A third repeat is not necessary. We anticipate performing dose finding studies on 2-4 lead compounds. Each study will require 54 mice. **In total, this subaim will require 432 mice.**

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
432		432

Aim 1.d.B. In vivo dose finding for lead molecules in MERS-CoV efficacy studies. Dose finding studies for compounds to be assessed against MERS-CoV will be executed in exactly the same way as those for SARS-CoV except for the following two differences:

Mice: MERS-CoV studies must be performed in C57BL/6 mice genetically modified to express a humanized dipeptidyl peptidase-4 (hDPP4) that were created and are bred at UNC Chapel Hill.

Virus: A dose of 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) will be delivered intranasally in 0.05ml.

Repetition: Similar to the SARS-CoV studies above, **these studies will require 432 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
432		432

Aim 1.d.C. Prophylactic and Therapeutic Efficacy of Lead Molecules with SARS-CoV challenge.

Rationale: After we determine a prophylactic dose regimen for a lead that provides a robust antiviral effect against SARS-CoV, we aim to determine if therapeutic dosing will also provide an antiviral effect if given during an ongoing SARS-CoV infection. As a control, we will include the prophylactic dosing arm proven to be efficacious above.

Drug Dosing: The dose, route and frequency will be determined in PK and dose finding studies above. Prophylactic dosing will begin 1 day before infection while therapeutic dosing will begin 1 day after infection.

Mice: Equivalent numbers of male and female 20-25 week old C57BL/6J (Jackson Labs) will be used for these studies. The four treatment groups will be as follows: prophylactic vehicle or compound, and therapeutic vehicle or compound. Each treatment group will be comprised of 12 mice (6 male/6 female).

Virus: A dose of 1E+04 PFU SARS-CoV MA15 strain (mouse adapted) will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi, animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study will require 48 mice. This study will be repeated three times in order to confirm data is consistent and concordant (144 mice/compound). We anticipate performing efficacy studies on 2-4 lead compounds for a **total of 576 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
576		576

Aim 1.d.C. Prophylactic and Therapeutic Efficacy of Lead Molecules with MERS-CoV challenge. Efficacy studies for compounds to be assessed against MERS-CoV will be executed in exactly the same way as those detailed for SARS-CoV except for the following two differences:

Mice: MERS-CoV studies must be performed in C57BL/6 mice genetically modified to express hDPP4 that were created and are bred at UNC Chapel Hill.

Virus: A dose of 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) will be delivered intranasally in 0.05ml.

Repetition: Each study will require 48 mice. This study will be repeated three times in order to confirm data is consistent and concordant (144 mice/compound). We anticipate performing efficacy studies on 2-4 lead compounds for a **total of 576 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
576		576

Aim 1.d.D. Dose finding for lead molecules in SARS-or MERS-CoV efficacy studies in aged animals
Rationale: Aged animals are a most stringent model within which to test efficacy of antivirals. Given that both SARS- and MERS-CoV disease is exacerbated in older humans, aged mice serve to model this aspect of CoV pathogenesis. Input doses of virus will have to be decreased in these models given the sensitivity of aged mice to these infections.

Drug Dosing: The formulation, route and frequency will be determined above in younger mice.

Mice: Equivalent numbers of male and female 1.5 year old C57BL/6J (SARS-CoV, Jackson Labs) or 1.5 year old C57BL/6 hDPP4 (MERS-CoV) will be used for these studies. Each study will have four infected and treated groups: High, medium and low compound, and a vehicle control. Each treatment group infected with SARS-CoV will be comprised of 12 mice (6 male/6 female). To control for potential effects associated with dosing on animal weight or pulmonary function, a smaller “sham” infected group will also be included (n = 6).

Virus: For SARS-CoV, a dose of 1E+02 or 1E+03 PFU SARS-CoV MA15 strain (mouse adapted) or MERS-CoV P35C4 (mouse adapted) will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi (days post infection), animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Since this study is simply intended to provide a prophylactic dose exhibiting an antiviral effect for future studies, upon repeating Experiment 1, not all doses may be required. A third repeat is not necessary. We anticipate performing dose finding studies on 2-4 lead compounds. Each study per virus will require 54 mice. **In total, this subaim will require 864 mice.**

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
864		864

Aim 1.d.E. Prophylactic and Therapeutic Efficacy of Lead Molecules with SARS-CoV or MERS-CoV challenge in aged mice
Rationale: After we determine an optimal prophylactic dose is determined above, we aim to determine if therapeutic dosing will also provide an antiviral effect if given during an ongoing severe CoV infection. As a control, we will include the prophylactic dosing arm proven to be efficacious above.

Drug Dosing: Determined above.

Mice: Equivalent numbers of male and female 1.5 year old C57BL/6J (SARS-CoV, Jackson Labs) or 1.5 year old C57BL/6 hDPP4 (MERS-CoV) will be used for these studies. The four treatment groups will be as follows: prophylactic vehicle or compound, and therapeutic vehicle or compound. Each treatment group will be comprised of 12 mice (6 male/6 female).

Virus: For SARS-CoV, a dose of 1E+02 or 1E+03 PFU SARS-CoV MA15 strain (mouse adapted) or MERS-CoV P35C4 (mouse adapted) will be delivered intranasally in 0.05ml. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi, animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study per virus will require 96 mice. This study will be repeated three times in order to confirm data is consistent and concordant (288 mice/compound). We anticipate performing efficacy studies on 2-4 lead compounds for a **total of 1,152 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
1,152		1,152

Aim 1.d.F. Therapeutic Efficacy of Lead Molecules with SARS-CoV or MERS-CoV challenge in Rag-/- mice

Rationale: To evaluate the capacity of EIDD-1931/2801 to clear virus in the absence of functional adaptive immunity, we will infect groups of 20 male and female C57BL/6 Rag1-/- (SARS-CoV) or C57BL/6 288/330 hDPP4 Rag1-/- (MERS-CoV) mice, which we recently generated.

Drug Dosing: Determined above.

Mice: 20 male and 20 female 20-week old C57BL/6 Rag1-/- (SARS-CoV) or C57BL/6 288/330 hDPP4 Rag1-/- (MERS-CoV) mice will be used for these studies. After 10 days of infection, each cohort will be split in half and either treated with vehicle or and effective therapeutic drug dose as determine above.

Virus: A dose of 1E+04 PFU SARS-CoV MA15 strain (mouse adapted) or 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) will be delivered intranasally in 0.05ml. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 15dpi, 5 animals per sex will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL. On 25dpi, the remaining animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study per virus will require 40 mice (80 mice for both SARS- and MERS-CoV. This study will be repeated three times per virus in order to confirm data is consistent and concordant (240 mice/compound). We anticipate performing efficacy studies on 2-4 lead compounds for a **total of 960 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
960		960

E.2. SPECIFIC AIM 2: *To define MOA of EIDD-1931/2801 and impact of resistance mutations on* in vivo *therapeutic efficacy, viral fitness, and pathogenesis.*

Aim 2.d. Determine the impact of resistance mutations on virus replication, fitness, and pathogenicity in mice infected with MERS-CoV and SARS-CoV.

Rationale: To determine mechanism of action, we aim to generate drug resistant virus through in vitro passage of CoV in the presence of a given lead compound. Rederived recombinant virus bearing putative resistance mutations will be evaluated for pathogenic potential as compared to its parent in vivo.

Mice: Equivalent numbers of male and female 20-25 week old mice will be used for all of these studies. C57BL/6J (Jackson Labs) will be used for SARS-CoV studies. MERS-CoV studies must be performed in C57BL/6 mice genetically modified to express hDPP4 that were created and are bred at UNC Chapel Hill. Each study will compare resistant virus to its respective wild-type parental strain. Each virus group will be comprised of 12 mice (6 male/6 female).

Virus: A dose of 1E+05, 1E+04, 1E+03 PFU SARS-CoV MA15 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. A dose of 1E+05, 1E+04, 1E+03 PFU MERS-CoV P35C4 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 7dpi, animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study will require 24 mice. This study will be repeated three times in order to confirm data is consistent and concordant (72 mice/resistant strain). We anticipate performing efficacy studies on 2-4 resistant viruses for SARS- (288 mice) and MERS-CoV (288 mice) for a **total of 576 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
576		576

Aim 2.e. Determine the impact of resistance mutations on therapeutic efficacy.

Rationale: To determine the susceptibility of drug resistant viruses to treatment, we will examine the impact of resistance mutations on treatment efficacy in vivo.

Drug Dosing: The dose, route and frequency will be determined in PK and dose finding studies above. Therapeutic dosing will begin 1 day after infection.

Mice: Equivalent numbers of male and female 20-25 week old mice will be used for all of these studies. C57BL/6J (Jackson Labs) will be used for SARS-CoV studies. MERS-CoV studies must be performed in C57BL/6 mice genetically modified to express hDPP4 that were created and are bred at UNC Chapel Hill. Each study will have four groups: wild-type virus with vehicle or compound, and resistant virus with vehicle or compound. Each group will be comprised of 10 mice (5 male/5 female).

Virus: A dose of 1E+03 PFU SARS-CoV MA15 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. A dose of 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi, animals will be sacrificed, lung hemorrhage scores will be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study will require 40 mice. This study will be repeated three times in order to confirm data is consistent and concordant (120 mice/resistant strain). We anticipate performing efficacy studies on 2-4 resistant viruses for SARS- (480 mice) and MERS-CoV (480 mice) for a **total of 960 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
960		960

E.3. SPECIFIC AIM 3. *To test combination regimens for treatment of emerging CoV disease.*

Aim 3.b. Evaluate multidrug efficacy in murine models of SARS and MERS.

Rationale: To determine if combinations of antivirals will provide an additive or synergistic antiviral effect, we will examine the impact of combination therapy on treatment efficacy in vivo. In addition, to determine if combination therapy more potently diminish resistant virus replication, we will examine the impact of combination therapy on treatment efficacy in mice infected with resistant virus created in Aim 2.

Drug Dosing: The dose, route and frequency will be determined in PK studies performed by collaborators in Aim 3.1. Therapeutic dosing will begin 1 day after infection.

Mice: Equivalent numbers of male and female 20-25 week old mice will be used for all of these studies. C57BL/6J (Jackson Labs) will be used for SARS-CoV studies. MERS-CoV studies must be performed in C57BL/6 mice genetically modified to express hDPP4 that were created and are bred at UNC Chapel Hill. For studies including remdesivir, mice lacking carboxylesterase 1c (*Ces1c*) will be used (C57BL/6 *Ces1c*$^{-/-}$ for SARS-CoV and C57BL/6 *Ces1c-/-* hDPP4 for MERS-CoV, Jackson Labs). For studies with wild-type virus, each study will have four groups: Vehicle, compound A, compound B and a combination of A and B. Each group will be comprised of 10 mice (5 male/5 female). For studies with wild-type and resistant virus, each study will have 8 groups: Vehicle, compound A, compound B and a combination of A and B for both wild-type virus and resistant. Each group will be comprised of 8 mice.

Virus: A dose of 1E+04 PFU SARS-CoV MA15 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. A dose of 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) or related resistant virus will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 5dpi, animals will be sacrificed, lung hemorrhage scores will be be recorded and lung tissues will be harvested for virus titer, pathology and Bioplex. Blood will also be taken for complete blood count and inflammatory cells isolated via BAL.

Repetition: Each study with wild-type mice will require 40 mice and will be repeated three times in order to confirm data is consistent and concordant (120 mice/combination evaluation). We anticipate performing efficacy studies on 2-4 combinations for SARS- (480 mice) and MERS-CoV (480 mice) for a **total of 960 mice**. Each study comparing wild-type and resistant virus treated with single drugs or combination will require 64 mice. We anticipate performing these studies for 2 resistant viruses for both SARS and MERS to be repeated three times for a **total of 768 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
1,728		1,728

Aim 3.c. Evaluate the therapeutic potential of co-administered lead CoV-active compounds and immunomodulators in SARS-CoV- and MERS-CoV-infected mice.

Rationale: Severe disease caused by both SARS- and MERS-CoV is in part driven by immunopathology. Immunomodulators like corticosteroids in isolation have not been successful in improving disease outcomes and can even lead to exacerbated disease. Moreover, steroids in isolation often lead to prolonged viremia which can lead to increased chances of transmission. Treatment of SARS-CoV with remdesivir (GS-5734) after 2dpi does not provide protection from disease yet significantly diminishes viral load. Thus, in this subaim, we propose to couple remdesivir with immunomodulators to more holistically treat disease by targeting both the virus and the excessive immune response.

Drug Dosing: Remdesivir will be delivered subcutaneously at 25mg/kg twice daily. As we have done extensively in the past, remdesivir will be solubilized in vehicle containing 12% sulfobutylether-β-cyclodextin in water (with HCl/NaOH) at pH 5.0. The corticosteroid, dexamethasone, will be administered intraperitoneally at a dose of 10mg/kg daily which has been shown to be protective in mice infected with influenza. A human equivalent dose of IFN-β (1.6 million units in PBS; R&D Systems) will be administered subcutaneously every other day. **Drug dosing in the initial study will begin on 1dpi.** The study outlined below will be similar for other anti-inflammatory agents that arise within our program or the U19. For example, as a backup, we will explore the utility of antiviral antibodies (S227.4, 25mg/day) or immune modulating antibodies like the T-cell depleting antibody CTLA4-Ig (400mg/day) will be administered via intraperitoneal injection once daily.

Drug dosing to determine the latest time in which we obtain a clinical benefit: If the initial study where we begin drug dosing on 1dpi demonstrates and increased protective effect, in subsequent experiments, we will determine if the treatment window can be extended by initiating combination therapy on 2, 3, 4, 5, or 6 dpi.

Mice: Equivalent numbers of male and female 20-25 week old mice will be used for all of these studies. For studies including remdesivir, mice lacking carboxylesterase 1c (*Ces1c*) will be used (C57BL/6 *Ces1c*$^{-/-}$ for SARS-CoV and C57BL/6 *Ces1c-/-* hDPP4 for MERS-CoV, Jackson Labs). Each study will have four groups: vehicles, vehicle/anti-inflammatory, remdesivir, and remdesivir/anti-inflammatory. Each group will be comprised of 10 mice (5 male/5 female).

Virus: A dose of 1E+04 PFU SARS-CoV MA15 strain (mouse adapted) will be delivered intranasally in 0.05ml. A dose of 1E+05 PFU MERS-CoV P35C4 strain (mouse adapted) will be delivered intranasally in 0.05ml. Virus will be diluted in PBS. Mice will be anaesthetized with a mixture of ketamine/xylazine prior to infection.

Endpoints: Animal weights will be recorded daily. Pulmonary function will be monitored daily via whole-body plethysmography. On 6dpi, animals will be sacrificed, lung hemorrhage scores will be recorded, and lung tissues will be harvested for virus titer and pathology.

Repetition: Each study with wild-type mice will require 40 mice and will be repeated three times in order to confirm data is consistent and concordant (120 mice/combination evaluation). We anticipate performing efficacy studies on 2-4 anti-inflammatory agents for SARS- (480 mice) and MERS-CoV (480 mice) for a **total of 960 mice**. In subsequent experiments we aim to extend the therapeutic window and delay treatment initiation. These studies will be similar to the initial studies except for the time of treatment initiation. Thus, for these studies, 40 mice will be required per study. We anticipate performing 5 studies per virus for a **total of 400 mice**.

Animal Numbers and Pain Categories

Total Animals	# Pain Category C	# Pain Category E
1,360		1,360

2. Justifications

This proposal aims to determine the efficacy and mechanism of action for lead antiviral molecules and combination therapies to treat SARS- or MERS-CoV replication and pathogenesis in rodents. The work described above will provide key proof of principle data. These studies cannot be done without vertebrate animals. There is no *in vitro* system that accurately mimics virulence of either CoV that is seen in animals or humans or that would accurately predict the outcome of infection. At this time, there is no substitute for in vivo efficacy studies, studies to assess drug metabolism and PK. Our studies are designed with the fewest number of animals while retaining statistical significance.

To determine antiviral efficacy of a drug candidate, it is essential that this be done within models that recapitulate the pathogenesis of the human disease the drug is intended to treat. The studies described above will be performed within SARS- and MERS-CoV pathogenesis models that were developed within the Baric lab. Importantly, both SARS- and MERS-CoV pathogenesis in these models is driven by high titer replication limited to the lung, severe end stage lung disease consistent with acute respiratory disease syndrome (ARDS), and measureable decreases in pulmonary function. Thus, both models employed herein accurately recapitulate many aspects of the human disease phenotypes and will provide essential information for the development of antiviral treatments targeting emerging CoV.

With this application, we will develop new antiviral therapies and these experimental lead molecules currently have unknown PK and safety profiles. Our collaborators at SR will attain this information if optimized leads are suitable for in vivo efficacy studies. We will be using two optimized antivirals with known PK and safety profiles. First, remdesivir (GS-5734) has been extensively studied in rodents and non-human primates. We have shown that prophylactic 25mg/kg remdesivir administered subcutaneously BID, can inhibit both SARS-CoV and MERS-CoV replication and pathogenesis and is well tolerated. Second, EIDD-1931 was well tolerated up to 1000 mg/kg QD in 7-day dose ranging toxicity studies and at 500mg/kg BID in mice efficacy studies. Moreover, the antiviral efficacy of EIDD-1931 in mice has been previously determined against multiple other viruses including chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV), Ebola virus, respiratory syncytial virus (RSV) and Influenza A virus strains and in all cases the drug was well tolerated.

3. Minimization of Pain and Distress

Mouse adapted strains of SARS- and MERS-CoV replicate efficiently in the lungs of mice and may produce significant disease in young and aged animals, including acute onset respiratory distress syndrome, a clinically devastating end stage lung disease with 50% mortality rates. Mice will be closely monitored daily for signs of clinical disease. Since analgesics may affect the outcome of infections, analgesics will not be used and we will rely on close monitoring and euthanasia of sick animals to prevent undue pain and suffering. In general, animals will be euthanized if they approach losing 30% of their starting weight; we recognize that this is a significant weight loss, but it is acceptable in these studies as some animals can recover from >25% weight loss after highly pathogenic coronavirus infection. We will euthanize moribund animals, regardless of weight loss criteria. For rodents, euthanasia will be performed by overdose with isoflurane. This will immediately be followed by organ harvest/exsanguinations, as prior treatment with these agents ensure that the animals will not suffer during this procedure due to operator error. This approach was chosen because unconsciousness and death occur quickly and the method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Criteria for euthanasia: Mice will be euthanized (isoflurane overdose followed by organ harvest) at the point at which they become moribund, lose over 20 percent of their starting weight (or up to 30 percent of their starting weight if the animals are being maintained under the weight loss exception), reach a clinical score of 4 or higher or reach pre-determined endpoints (ranging from 1 to 7 days post infection), whichever comes first.

0. No clinical signs
1. Ruffled fur.
2. Ruffled fur with hunched posture (only slight or no signs of dehydration)
3. As above with more severe signs of dehydration and some loss of body strength, some loss of spontaneous morbidity
4. Marked loss of spontaneous morbidity and pronounced dehydration
5. Moribund: unresponsive to stimuli and pronounced eye squint

-All scores except 5 can be qualified with a 0.5 for severity
-Any mouse that reaches a clinical score of 4 or more will be euthanized immediately

SELECT AGENT RESEARCH

Select Agent(s) to be used in the proposed research. [redacted] (b) (4), (b) (3) (A)

Registration status of all entities where select agent(s) will be used. [redacted] (b) (4), (b) (3) (A)

Facilities where the select agent(s) will be used. SARS-CoV will be manipulated in research activities at [redacted] (b) (3) (A), (b) (6)l, including establishment of viral replication curves and performance of plaque assays, in laboratory spaces that meet operational and procedural criteria for BSL-3 activities as outlined in the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 5th edition, as well as BSL-3 criteria outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016). In addition, all mouse studies at [redacted] (b) (3) (A), (b) (6) will be performed in an approved and registered BSL-3/ABSL-3 laboratory equipped with Techniplast SealsafeTM HEPA-filtered animal housing for rodents. All animal protocols will be approved by the [redacted] (b) (3) (A), (b) (6) IACUC.

The BSL-3/select agent laboratory is equipped with biosafety cabinets, incubators, centrifuges with containment features, cold storage units, an autoclave, sink, eyewash and life safety equipment, and mechanical system monitors and alarms to support effective isolation and containment of operations involving SARS-CoV. The anteroom to the laboratory houses PAPR charging stations, laboratory and safety supplies, and a changing area. For both the BSL-2 and BSL-3 select agent spaces, access to select agents is restricted by the door between the hallway and anteroom and the door between the anteroom and BSL-3 space, [redacted] (b) (3) (A), (b) (6) All select agent materials are stored in locked freezers and incubators.

Procedures for monitoring possession, use and transfer of select agents. All personnel who will have access to select agent-regulated materials have been added to the Select Agent registration following security risk assessments prescribed by the CDC Select Agent Program. Personnel have completed training in all aspects of select agent compliance requirements and have adopted changes to standard operating procedures as applicable to assure that these requirements are met. Personnel will follow all procedures prescribed for accessing and securing the laboratory, documenting laboratory activities and materials used, and responding to incidents that could result in theft, loss, or release of select agent-regulated materials. Transfers of select agent-regulated materials will be coordinated by the laboratory managers and Responsible Official in accordance with standard operating procedures, including obtaining appropriate permits for shipping select agent materials and observing all regulations for shipping, both under dangerous goods and select agent regulations. Transfer of select agent RNA in TRIzol from registered BSL-3 to registered BSL-2 space and cDNA from registered BSL-2 space to non-registered BSL-2 space is conducted according to current select agent rules, regulations, and guidelines, including the new inactivation policies released in 2017.

Biosafety, biocontainment, and security of the select agent(s). The laboratories have been operational with BSL-3 core policies and procedures for more than a decade. Standard operating procedures at BSL-3 have been reviewed and approved by the [redacted] (b) (3) (A), (b) (6) respective Institutional Biosafety Committees and undergo both annual review and approval as well as updates as laboratory processes change or biosafety procedures evolve. The content of these documents has been formatted to conform to select agent regulations for the biosafety, security, and incident response plans. Additionally, lab-specific security risk assessments have been completed and recommendations implemented to ensure that security measures and procedures are sufficient to effectively minimize the possibility of unauthorized access to select agent-regulated materials. The facilities at [redacted] (b) (3) (A), (b) (6) have undergone multiple CDC inspections and are currently in compliance with CDC requirements relating to SARS- CoV and select agent status.

Biocontainment resources. The BSL-3/select agent laboratory is under negative pressure, with redundant systems to ensure that negative pressure is maintained. The laboratory has autoclaves to decontaminate waste materials as well as approved protocols for treatment or inactivation of any materials leaving the laboratory. All personnel are extensively trained in basic virology and safety protocols before being approved for select agent work, and undergo additional extensive training to work with SARS-CoV as a BSL-3 pathogen. Annual testing is performed to verify that biosafety cabinets, laboratory supply/exhaust systems (including alarms), and other laboratory equipment are functioning as designed. The laboratory is secured at all times, and only personnel who have successfully completed Select Agent clearance and laboratory specific training requirements are permitted to enter without an escort.

MULTIPLE PI LEADERSHIP PLAN

Dr. Denison will serve as the corresponding PI and [redacted] as co-PI in this multi-investigator application. Both PIs have been involved in every step of planning and writing this grant and will continue to be involved in all subsequent planning and decision-making required to carry out the aims. We have chosen to submit this as a multi-investigator application due to the longstanding collaboration between our two labs and complementary nature of the expertise of the two PIs. The combination of these two labs fits well with the logical structure of aims presented in this application. Representatives at the Emory Institute for Drug Development (EIDD) and [redacted] [redacted] will serve as consultants on this proposal, and Southern Research (SR) with provide additional medicinal chemistry expertise.

Collaboration. [redacted] labs have been collaborating for over 15 years, with ongoing very productive interactions. Early on, the collaboration resulted in development of a reverse genetics system for mouse hepatitis virus (MHV), the first successful reverse genetics system for coronaviruses (CoVs). This breakthrough led to significant progress in the field of CoV biology. Building on the methodology developed for MHV, severe acute respiratory syndrome (SARS-CoV) reverse genetics was in place and published (Yount et al., 2003, PNAS) less than six months following identification of SARS-CoV as the etiologic agent of the 2002-03 SARS epidemic. This rapid development facilitated numerous research projects aimed at better understanding a newly emerged human CoV causing severe disease with pandemic potential. These accomplishments similarly enabled prompt establishment and publication (Scobey et al., 2012, PNAS) of a reverse-genetics system for Middle East respiratory syndrome (MERS)-CoV, the causative agent of MERS, less than a year following initial report of MERS-CoV as a novel agent of fatal pneumonia. Reverse genetics for other CoVs also has been developed based on the strategy devised by our two groups. Drs. Denison and [redacted] continue to collaborate on a number of projects, including the current funding cycle of the AD^{3}C CETR award, and the partnership has resulted in >20 co-authored published or submitted papers in many high-impact journals, such as PNAS, Nature Medicine, and Science Translational Medicine.

Contributions to current proposal. [redacted] has performed initial screening of hit compounds described in this proposal in continuous cell lines. Decades of experience applying reverse genetic approaches to elucidate mechanisms and determine fidelity of CoV replication has been applied to better understand antiviral mechanisms of action through generation and characterization of remdesivir and EIDD-1931 resistant CoVs. [redacted] has determined the efficacy of hit and lead compounds in primary human airway cells, and determined the efficacy of remdesivir in mouse models of CoV disease as part of the current funding cycle of the AD^{3}C CETR award. These results form the basis of the proposed research, a logical extension of findings made during the initial funding period and further building on synergistic strengths of the investigators.

Expertise. The Denison group discovered [redacted] proofreading and has significant expertise using in RNA-Seq to measure CoV replication fidelity. [redacted] has a long history using synthetic biology, reverse genetics, metagenomics, primary human lung cells and robust small animal models to study virus and host interactions of human and zoonotic CoVs. The VUMC and UNC groups in collaboration with industry partners (EIDD, [redacted], and SR) have pioneered a suite of methods and techniques that facilitate the comprehensive evaluation of CoV-targeting antivirals.

Communication between the principal investigators. Drs. Denison and [redacted] will communicate by phone once a month, or more often if needed, to review progress in each area of the project and to discuss plans for continued work. They will cooperatively make any decisions affecting global project direction. Drs. Denison and (b) (3) (A), (b) already frequently communicate as a result of their collaboration on this and other programs. Other members of the Denison and (b) (3) (A), (b) (6), including (b) (3) (A), (b) (6) communicate on a regular basis regarding experimental planning and results. We have established and will maintain monthly joint teleconferences between VUMC, UNC, EIDD, (b) (3) (A), (b) (6) and SR to share data, obtain critical analysis of go / no-go milestones, review progress toward IND for EIDD-1931, and discuss whether combination approaches meet criteria for continuation. Drs. Denison and (b) (3) (A), (b) and members of their laboratories attend at least one annual scientific meeting together, and face-to-face meetings are always incorporated at these venues. In addition, travel to one or the other lab will be planned as needed to ensure project progress and/or in situations where key personnel are unable to co-attend a scientific conference. Both laboratories are select agent-registered, and materials related to this application will be shipped between labs according to governing CDC and local institutional protocols.

Budget allocations. Drs. Denison and (b) (3) (A), (b) have submitted separate budgets and justifications to support the work performed in each of their respective laboratories. The budgets include travel to annual scientific meetings or the other laboratory.

Conflict resolution. Dr. Denison will have primary responsibility for resolving any conflicts that might arise among personnel involved with this project. He will obtain consultation and advice from VUMC senior faculty with no stake in the disagreement. All parties involved in any potential conflict will communicate directly with Dr. Denison, and he will meet with them confidentially as needed to resolve underlying problems. If conflicts cannot be resolved, senior colleagues of each PI at their respective institutions, with whom they have established working relationships and who are not directly involved in this project, will be asked to formally consult on the issue. These include (b) (3) (A), (b) (6), M.D. (b) (3) (A), (b) (6) and (b) (3) (A), (b) (6)Chair in Pediatrics) and (b) (3) (A), (b) (6). Conflicts not resolved in this manner will be discussed with appropriate officials at VUMC and UNC-Chapel Hill Offices of Research.

Studies with SARS-CoV, biosafety, and select agent status. The project incorporates experiments in the BSL-2 model system, MHV, and in SARS-CoV, which is both a biosafety BSL-3 agent and a biosecurity select agent. Both labs have approved protocols for safe work with MHV at BSL-2 and SARS-CoV at BSL-3. VUMC and UNC are approved select agent institutions, the programs are in compliance with select agent regulations, and the investigators are approved for work in select agent labs with SARS-CoV. (b) (3) (A), (b) (6) have long-established processes for movement of materials between BSL-3 labs. Those include communication with the Select Agent Program concerning any planned shipments.

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(b) (4)

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Resource Sharing Plan.

Data sharing. We are strongly committed to data sharing. In the event that any publications originating primarily from cell culture or *in vivo* experiments contain gene expression data, it will be standardized according to current conventions and deposited in public-access databases—i.e., MIAME-compliant and deposited in GEO. Any quantitative sequence analysis will be entirely open source and/or deposited in dBGaP so that results and analytical procedures are also publicly available. Links to our data will be included in any and all publications.

The primary method of data sharing is through peer-reviewed publications in scientific journals and by presentation at scientific meetings. In addition, data and results created from NIH-supported research will be submitted to NIH in annual progress reports per terms and conditions of this award.

Reagent sharing. To share resources with the academic research community, we will use the uniform Material Transfer Agreement (MTA), which basically acknowledges that the materials are proprietary to VUMC and permitting their use in a manner consistent with the Bayh-Dole Act and NIH funding requirements, which specify that research be made available to the scientific community and public.

Intellectual property. Intellectual property agreements, identified during the course of this project, will be accomplished by negotiation in good faith among the institutions and inventors. Any intellectual property discussions will take place with all key personnel present. VUMC Office of Technology Transfer and Commercialization and the UNC Office of Commercialization and Economic Development will assist the inventors in the production of necessary documents, working with the particular institutions, legal firms, and commercial interests. It is anticipated that companies and institutions will have access to these reagents by MTA (for research purposes) or by a license agreement to be negotiated in good faith with a company.

Sharing model organisms. Novel cell lines generated during the course of this project will be shared under appropriate MTAs or other governing agreements if requests for such materials are received. Model cell lines and any useful unique reagents (cDNAs, vector constructs, etc.) that are generated and reported in publications have been, and will be, made fully available to all reasonable requestors in the scientific community.

Authentication of Key Biological and/or Chemical Resources.

Cell authentication. Experiments employ human lung primary cells and immortal cell lines from mice, non-human primates, and humans.

Immortal lines are acquired form the ATCC or the UNC Lineberger Cancer Center's Tissue Culture Facility (TCF). The TCF maintains cell lines, utilizing short terminal repeat (STR) marker profiling for authentication. New cell lines not available directly from the ATCC or TCF can be authenticated through the STR marker service provided by the TCF. Cell lines are routinely evaluated for *Mycoplasma* contamination. When receiving cell lines, lab members maintain them in isolation from other authenticated cell lines until *Mycoplasma* testing and STR marker profiling are performed. All cell lines must be authenticated before commencing experimental work. Records are maintained for each cell line regarding 1) origin, 2) date of resuscitation, 3) number of passages, 4) all test results, 5) any distinguishing growth behavior; and 6) any known genetic features. Cells that have been passaged for six months after receipt or from resuscitation will be re-authenticated, or a new vial of working stock will be thawed. Lab members routinely examine cultured cell morphology by phase microscopy and monitor growth characteristics. New vials of working stock are thawed if deviation from baseline is observed. *Mycoplasma* contamination is re-checked whenever cells are extensively passaged to create new stocks.

Early passage primary lung cells from humans (i.e., human airway epithelial cultures) are a key reagent for the proposed studies. Human cells are derived from donors of both sexes and from all ages and ethnic groups. Care is taken during cell isolation to only handle one human organ at a time. Similarly, primary cell populations are handled carefully—only one donor cell type from a single donor at a time—to avoid any mixing. Cultures are observed for well-described prototypical characteristics of human primary lung cells. For quality control, cells are cultured in antibiotic-free media to detect any potential microbial contamination and are subjected to *Mycoplasma* testing. Once airway epithelial cells are grown as polarized and differentiated monolayers, a representative sample is subjected to quality control histological analysis and STR marker profiling by the TCF.

Animal (mice) authentication. Inbred mouse strains are an invaluable tool for biomedical research and represent a key aspect of this proposal. To ensure that genetic backgrounds of all mice used within this program are known and, when applicable, part of known inbred strains, we will genotype each mouse strain on the appropriate MUGA platform (PMC4751547). The most recent iteration of this state-of-the-art genotyping array contains over 140,000 markers and can be used to precisely determine the genetic background at the substrain level and precise location (at <1 megabase resolution) of genomic regions derived from different inbred mouse strains. In this way, the identity and genomic integrity of all mice used within these studies will be ensured. As new diagnostic assays become available, we will assess their utility and cost effectiveness comparted to MUGA arrays and implement alternatives as appropriate. Furthermore, for each mutant mouse strain used within the project, relevant mutation(s) will be assessed for each cohort of experimental animals with a validated diagnostic PCR assay or Sanger sequencing to ensure reliable results.

Virus authentication. Mutant and wildtype viruses contain unique genetic signatures that allow for distinguishing strains and mutation profiles using a combination of full-genome sequencing and RT-PCR +/- restriction fragment length polymorphism analysis. We have developed defined primer pairs to accurately discriminate among MHV, SARS-CoV, and MERS-CoV. Genotypes of all viruses, including those recovered from infectious clones, will be confirmed prior to use. Likewise, identify of frozen virus stocks will be confirmed and certified free of contaminating viruses prior to experimentation.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0636907050000

Legal Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department:
Division:
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave South, (b) (3) (A), (b) (6)
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	(b) (3) (A), (b) (6)		(b) (3) (A), (b)	

Position/Title:
Street1*: 1720 2nd Avenue South, (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6) Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 2 - Novel Therapeutics for Emerging Alphavirus

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	the University of Alabama at Birmingham
Duns Number:	0636907050000
Street1*:	1720 2nd Ave South, AB1170
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	352940111
Project/Performance Site Congressional District*:	AL-007

Project/Performance Site Location 1

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Oregon Health & Science University
DUNS Number:	0969975150000
Street1*:	Vaccine and Gene Therapy Institute
Street2:	505 NW 185th Ave, Mail Code VGTI
City*:	Beaverton
County:	Washington
State*:	OR: Oregon
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	97006-3448
Project/Performance Site Congressional District*:	OR-001

Project/Performance Site Location 2

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Oregon Health & Science University
DUNS Number: 0969975150000
Street1*: 3181 SW Sam Jackson Park (b) (3) (A), (b) (6)
Street2:
City*: Portland
County: Multnomah
State*: OR: Oregon
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 97239-3098
Project/Performance Site Congressional District*: OR-003

Project/Performance Site Location 3

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: the University of North Carolina at Chapel Hill
DUNS Number: 6081952770000
Street1*: Administrative Office Building (AOB) CB# 1350
Street2: 104 Airport Dr (b) (3) (A), (b) (6)
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-1350
Project/Performance Site Congressional District*:

Project/Performance Site Location 4

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Colorado Denver
DUNS Number: 0410963140000
Street1*: Mailstop F428, Anschutz MEdical Campus
Street2: Building 500, 13001 East 17th Place, Rm (b) (3) (A), (b) (6)
City*: Aurora
County:
State*: CO: Colorado
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 80045-2571
Project/Performance Site Congressional District*:

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_2_Project_Summary_UAB.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project2_NovelAlphavirusAntivirals_references.pdf
10.Facilities & Other Resources	Facilities_Alpha.pdf
11.Equipment	Equipment_Alphavirus.pdf
12. Other Attachments	Product_Development_Strategy.pdf

PROJECT SUMMARY

Mosquito-transmitted alphaviruses cause outbreaks of incapacitating acute and chronic arthritis and life-threatening encephalitis. The arthritogenic alphaviruses include the re-emerging chikungunya (CHIKV), Mayaro, and Ross River viruses. Since 2004, CHIKV has infected millions of people and expanded into Europe, Asia, and Americas. As arthritis can endure for months to years after infection with an arthritogenic alphavirus, large epidemics have severe economic consequences. The encephalitic alphaviruses include Eastern, Venezuelan (VEEV), and Western equine encephalitis viruses that are endemic to the Americas and infection can lead to mortality or long-term neurological sequelae. There are currently no approved alphavirus-specific vaccines or antiviral agents. In collaboration with Southern Research, we used HTS to identify non-toxic small molecules that inhibit CHIKV and VEEV replication. Additionally, we identified chemically distinct compound classes that display broad inhibitory activity against alphaviruses, as well as other pathogenic human viruses (e.g., flaviviruses). We also mapped resistance mutants against two highly active compounds and identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as potential antiviral targets. In addition to this work, in collaboration with the Emory Institute for Drug Development, we identified novel nucleosides, which target RNA-dependent RNA polymerases, with potent antiviral activity against alphavirus infection. Thus, we have identified potent antiviral compounds against three distinct molecular targets essential for alphavirus replication. The goal of this highly interactive Project 2 of the Antiviral Drug Discovery and Development Center (AD$_3$C) is to develop new therapeutic strategies that inhibit alphavirus replication, prevent selection for drug resistance, and reduce alphavirus disease severity. In ***Specific Aim 1***, lead compounds will be optimized in collaboration with Core A and Core B by iterative medicinal chemistry, cell culture-based antiviral and cytotoxicity assays, and *in vivo* pharmacokinetic studies to improve their efficacy, selectivity, solubility, and bioavailability. In ***Specific Aim 2***, we will work with the other AD$_3$C Projects to define the breadth of antiviral activity and cell type specificity, molecular targets through resistance mapping and structural/computational analyses and identify synergy profiles for compounds with potent activity. In ***Specific Aim 3***, optimized compounds will be evaluated for *in vivo* efficacy using well-established pre-clinical mouse models of alphavirus infection. Since therapies that target different aspects of the viral life cycle are likely to show improved efficacy and limit the development of drug resistance, combination therapies also will be tested. Last of all, since alphavirus-induced disease includes inflammatory immune pathology, we will evaluate the therapeutic potential of combining antiviral therapy with anti-inflammatory regimens. This work will promote the development of new antiviral therapeutic strategies that have the potential to improve human health.

FACILITIES & OTHER RESOURCES – [redacted]

(b) (6), (b) (3) (A)

[redacted]

(b) (3) (A), (b) (6)

(b) (4), (b) (3) (A)

[redacted]

(b) (3) (A), (b) (6)

[redacted]

(b) (3) (A), (b) (6)

[redacted]

(b) (4), (b) (3) (A)

[redacted]

(b) (3) (A), (b) (6)

[redacted]

FACILITIES AND OTHER RESOURCES

(b) (4), (b) (3) (A)

[redacted]

(b) (4), (b) (3) (A)

[redacted]

(b) (4), (b) (3) (A)

[redacted]

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Introduction

As required by this RFA, outlined below are the key milestones and associated timelines for the Antiviral Drug Discovery and Development Center (AD3C) of which Dr. Richard Whitley is the Program Director. The theme is to *develop new small molecule therapeutics for emerging and re-emerging viral infections by inhibiting viral replication.* Activities will include the identification of novel chemical scaffolds and the application of basic virology research activities to elucidate mechanisms of action for identified lead molecules that inhibit viral replication. Medicinal chemistry and lead development will advance identified compounds through the drug discovery and development pathway, ultimately leading to preclinical evaluation of drug candidates to identify the most promising compounds for clinical evaluation. The AD3C has four interrelated research **Projects**, with a focus on coronaviruses, alphaviruses, flaviviruses and influenza. These **Projects** will be supported by three Cores: an Administrative Core (**Core A**), an Assay Core (**Core B**) and a Medicinal Chemistry and Lead Development Core (MCLDC; **Core C**). Organization and interaction between all **Projects** and **Cores** will be orchestrated by the Administrative Core, with day-to-day AD3C operations led by Adminstrative Core personnel. An Executive Committee (EC) consisting of all Project and Core Leads will regularly review AD3C activities and productivity. Finally, an external Scientific Advisory Committee (SAC) will be established to provide a high-level evaluation of the progress of the AD3C annually (with regular reports for **Go/No-Go** decisions), and to aid in refining AD3C activities and direction. Unique to the proposed research is that in addition to identifying lead compounds for particular viruses such as dengue or chikungunya virus, our approaches will identify 1) mechanisms and targets associated with viral replication; 2) assessment of resistance; 3) due to close interaction among **Projects**, compounds with activity against multiple virus families; and 4) analysis of the potential for combination therapies predicated on the use of nucleoside analog-based inhibition of viral polymerases paired with inhibitors of other steps in viral replication or immunopathogenic host responses. These studies provide multiple opportunities to fulfill the goals outlined in the RFA.

Approach

The four **Projects** working with the Assay Core (**Core B**), Medicinal Chemistry and Lead Development Core (MCLDC; **Core C**) and the Administrative Core (**Core A**) will advance compounds through biological and biochemical evaluation of compounds, medicinal chemistry, lead optimization and *in vivo* ADME and efficacy studies. Each project will pursue the goal of identifying a lead series of compounds as well as a backup series to ensure success. The overarching objective is to find broad spectrum antiviral compounds for the treatment of diseases caused by emerging viral pathogens. The AD3C is uniquely qualified to do so, based upon the strategies outlined in the proposals, the team of scientists assembled, and the experience in collaborative drug discovery between Southern Research (SR), Gilead Sciences, the Emory Institute of Drug Development (EIDD), and respective scientists at the participating institutions. The basic science and mechanistic studies will be conducted by the four **Projects**. The three cores will coordinate the evaluation, lead optimization, structure-activity relationship identification, pharmacokinetic evalution (both *in vitro* and *in vivo*), and toxicology studies. The exact profile of the emerging lead compounds will vary and be driven by the scientific studies. However, to ensure that the AD3C can identify compounds with the best overall profile and the greatest chance of succeeding, all of the data will be available to all researchers using the collaborative software, Citrix ShareFile.

Selection of an IND Candidate

The AD3C will utilize a pharmaceutical company model for discovery and development. In doing so we have established key attributes that determine advancement of an investigated compound to an IND candidate, which we will use as a guide in our preclinical studies. These include:

Synthesis

- No structural alerts or reactive groups
- No issues in scale up for GLP toxicity and Phase I studies
- No impurity issues

- No significant stereochemistry issues
- No obvious cost of goods issues

Biopharmaceutics

- Crystalline, MP >125, non-hygro
- Bioavailability and oral PK completed with crystalline compound
- Solubility, PK and dose compatible with standard immediate release formulation
- Stability profile compatible with delivery options
- Required delivery options defined and realistic, according to disease target (i.e., pneumonia versus encephalitis)

Absorption

- Conform with Lipinski's Rule of Five
- Predicted human intestinal absorption will be adequate to achieve predicted efficacious human exposure and fraction absorbed ≥30%
- Exposure in dog or monkeys using single PO dose attaining exposure equivalent to predicted human clinical efficacious exposure

Clearance

- The predicted human PK for the compound will be assessed using *in vitro* parameters such as human liver microsome data and *in vivo* animal data
- Major CL mechanisms investigated in animals and predicted in humans including ID of likely CYPs and other metabolizing enzymes
- Variability in metabolic clearance assessed
- Projected human total CL <10 ml/min/kg
- Projected oral bioavailability >30%
- Metabolic Drug-Drug Interaction (DDI): Compound as cyp inhibitor assessed

PK/PD and Dose Prediction

- The *in vivo* relationship between drug concentration and pharmacodynamic response over time has been characterized and the human clinical pharmacology predictions relating to dose (frequency and amount) and onset, duration and magnitude of response fall within the criteria defined in the product profile

Distribution

- Plasma or serum protein binding compared in human and pharmacology and tox species; blood to plasma ratio determined
- For CNS-targets only: not a substrate for P-gp

Pharmacology

- Target Identified
- Defined mechanism of action
- Human target used for profiling
- The potential impact of human target polymorphisms has been considered.
- Potency differences between human target and species used in efficacy models is understood to guide human dose predictions
- Greater than 100x selectivity vs. relevant human targets

Safety

- Ames – negative *in vitro* test for bacterial mutagenicity
- Appropriate preclinical tox species selected
- Broad ligand profiling completed using standardized CEREP panel
- <50% inhibition of HERG, Nav1.5, LQT1, and L-type current at 100x the projected efficacious free-plasma levels in humans.

Intellectual Property

- Appropriate patent protection is in place for both the lead and backup series

These parameters will assist the AD3C to make decisions on an ongoing basis throughout the project.

In addition, for this Project (alphaviruses) in particular, we have divided the drug discovery process up into several key measurable milestones (see below) and allocated the amount of time that will be needed to accomplish each goal (**Figure 1**). These timelines were established based upon past experience and industry standards and are set to identify an IND candidate(s).

Key Milestones

1. Lead compound optimization (12 months)
2. Define spectrum of antiviral activity (6 months)
3. Define resistance and mechanism of action (6 months)
4. *In vivo* efficacy with proof of concept compound (12 months)
5. Preclinical candidate and studies to enable potential IND filing (12 months)
6. Preclinical Candidate (13 months)
7. Candidate Selection and IND Enabling Studies (12 months)
8. IND Preparation (3 months)

Each of these milestones has a number of associated studies and decision points that are listed below and outlined in Figure 1.

Details

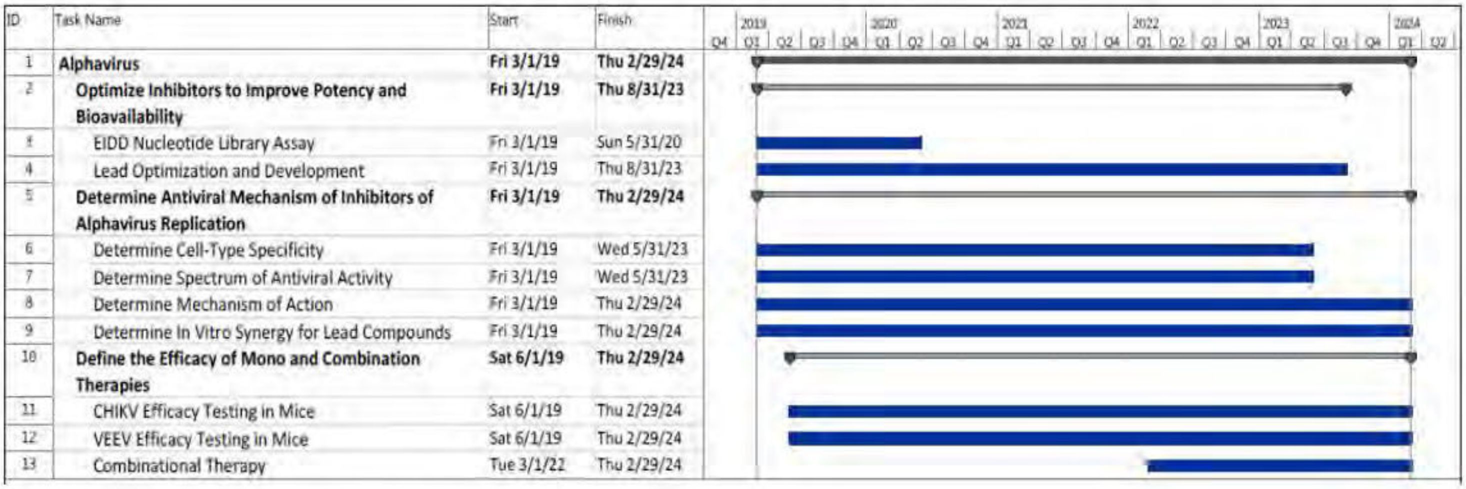
1) Lead Compound Optimization (12 months)
    a. Assays to evaluate compound efficacy validated and testing of novel scaffolds completed
    b. Selectivity in secondary assays
        i. Compounds with potency (EC_{50}<1μM) and low cytotoxicity (>10 fold EC_{50})
    c. Chemistry triage identifies multiple chemical series with potency and selectivity
2) Define spectrum of antiviral activity (12 months)
    a. Determine compound activity across alphavirus species including arthritogenic (Old World) and encephalitic (New World) alphaviruses
    b. Define compound antiviral activity and cytotoxicity across cell types (human, insect, mouse cells)
3) Define resistance and mechanisms of action
    a. Identify resistance mutations by deep sequencing of genomes
    b. Introduction of resistance mutations into wild type infectious clone
    c. Testing of compound resistance against mutant phenotypes
    d. Time of addition studies, viral RNA/protein synthesis analysis, viral binding/assembly/release assays
    e. Structural and computational analyses
4) *In vivo* efficacy with proof of concept compound (12 months)
    a. Potency of < 0.5 μM against viral replication
    b. Tractable synthesis

   c. Oral bioavailability of >30% in mice
   d. No effect at 10μM in a general receptor profiling panel
   e. Synthetic feasibility determined (availability of starting materials, number of steps, time lines to produce 25 -50 grams) and freedom to operate analysis completed
5) Preclinical candidate and studies to enable potential IND filing (12 months)
   a. Lead series selected with cell-based activity
   b. Back-up series identified
   c. Robust structure activity demonstrated, mechanism understood, potency
   d. No hERG activity
   e. Solubility (≥10 μM), microsomal stability (>60% remaining at 30 minutes), measureable plasma free fractions (>1%) and an assessment of permeability (CaCo2)
   f. Bioavailability such that plasma concentration are 5-10 fold above EC_{50}
   g. Oral bioavailability of >20% in rodents preferred
6) Preclinical Candidate (13 months)
   a. *In vitro* antiviral activity and selectivity versus normal cells. Ranges for antiviral potency would be typically < 0.1μM and > 20 μM versus normal cells.
   b. Structure-activity relationships versus a primary endpoint indicating antiviral activity or mechanism
   c. Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors (no activity at 10μM)
   d. Compound stability as a dry powder and in solution at a range of pH's
   e. No inhibition of cytochrome P450's 10 μM (3A4, 2C9,2C19)
   f. Expanded PK studies including a second species (preferably dog). A plasma half-life of >2 hr (mouse or rat) and >30% bioavailability). Metabolite identification in vivo.
   g. Oral activity in a relevant mouse model of infection. No observable in vivo side-effects. CNS exposure if relevant (ED_{50} of <100 mg/Kg)
   h. Understanding the metabolic pathways involved using rodent, dog and human microsomes and hepatocytes as well as in vivo in rodents and dogs prior to toxicology studies
   i. Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies
   j. A 7-day non-GLP toxicity study in rodents (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))
   k. Predicted human dose of ≤500 mg per day.

7) Candidate Selection and IND Enabling Studies (12 months)
   a. GMP Manufacture of 1Kg of drug substance
      i. Stability studies to support tox
   b. Formulation development
   c. 14-day GLP toxicity study in rodents and dogs
   d. Cardiovascular assessment

8) IND Preparation (3 months)

These timelines are estimates and can vary depending upon compounds availability, resources and capacity at GMP manufacturing facilities. The majority of the antiviral activity assays will be conducted at Southern Research, with secondary screens and *in vivo* models run at OHSU, UC Denver or UNC. Importantly, SR's GLP facilities are available for later IND enabling studies. Since SR has already identified compounds with activity against VEEV we will be able to initiate secondary assays in FY1.



ID	Task Name	Start	Finish
1	**Alphavirus**	**Fri 3/1/19**	**Thu 2/29/24**
2	**Optimize Inhibitors to Improve Potency and Bioavailability**	**Fri 3/1/19**	**Thu 8/31/23**
3	EIDD Nucleotide Library Assay	Fri 3/1/19	Sun 5/31/20
4	Lead Optimization and Development	Fri 3/1/19	Thu 8/31/23
5	**Determine Antiviral Mechanism of Inhibitors of Alphavirus Replication**	**Fri 3/1/19**	**Thu 2/29/24**
6	Determine Cell-Type Specificity	Fri 3/1/19	Wed 5/31/23
7	Determine Spectrum of Antiviral Activity	Fri 3/1/19	Wed 5/31/23
8	Determine Mechanism of Action	Fri 3/1/19	Thu 2/29/24
9	Determine In Vitro Synergy for Lead Compounds	Fri 3/1/19	Thu 2/29/24
10	**Define the Efficacy of Mono and Combination Therapies**	**Sat 6/1/19**	**Thu 2/29/24**
11	CHIKV Efficacy Testing in Mice	Sat 6/1/19	Thu 2/29/24
12	VEEV Efficacy Testing in Mice	Sat 6/1/19	Thu 2/29/24
13	Combinational Therapy	Tue 3/1/22	Thu 2/29/24

Figure 1: Timeline of activities in Project 2 of AD3C

Product Development Plan

Introduction

The overall aim of **Project 2** is to identify novel anti-viral therapies for the treatment of alphavirus infection. We will prioritize compounds that inhibit chikungunya (CHIKV) and Venezuelan equine encepahlaitis (VEEV) viruses with emphasis on compounds that inhibit replication of other arthritogenic and/or encephalitic alphaviruses, and potentially members of other virus families as well. To do so, a series of **Go/No-Go** decisions have been established to facilitate obtaining (an) orally active drug(s). Described below are the criteria required to identify lead compounds suitable for animal models testing, the selection of a preclinical compound with the efficacy and safety profile to advance into non-GLP studies, additional metabolic profiling and pharmacokinetic studies. Synthetic feasibility also will be considered. These studies will enable the team to select a compound for IND enabling GLP studies leading to a phase I clinical candidate(s).

1. Intended use/indication of the proposed product and the biodefense/public health gap the product is intended to fill

Alphaviruses are clinically important RNA viruses transmitted to humans and other mammals through arthropod bites. They comprise approximately 30 species organized into 7 complexes based on antigenic and genetic analysis. The effects of alphavirus infection on human hosts range from generalized fever and malaise to debilitating neurological or rheumatic disease and death. In addition, due to the aerosol infectivity of some alphaviruses, they also are of biodefense importance. Arthritogenic alphaviruses include chikungunya virus (CHIKV; NIAID category C pathogen), Mayaro virus, o'nyong-nyong virus, Ross River virus (RRV), and Sindbis virus (SINV). Encephalitic alphaviruses include Eastern, Western, and Venezuelan equine encephalitis viruses (EEEV, WEEV, VEEV; NIAID category B pathogens). Arthritogenic alphaviruses most commonly cause febrile illness with incapacitating polyarthritis/polyarthalgia that can persist for months to years after cessation of viremia. Encephalitic alphaviruses are primarily associated with neurological disease including encephalitis and meningitis. Notably, severe complications of infection with either arthritogenic or encephalitic alphaviruses is common in susceptible populations including the very young, the aged, and the immunosuppressed.

Alphaviruses have demonstrated capacity to spread rapidly, and the global impact of alphaviral disease has increased dramatically in recent years. Since 2004, CHIKV has infected millions of people and expanded into Europe, Asia, the Pacific region, and the Americas. As chronic CHIKV disease is debilitating, large epidemics have severe economic impact. RRV, which causes ~4,000–7,000 cases in Australia and Papua New Guinea annually, has similarly caused explosive outbreaks, spreading from Australia to multiple islands in the Pacific Region including Fiji, the Cook Islands, and America Samoa. Moreover, the recent detection of a locally-acquired Mayaro virus infection, typically found only in tropical regions of South America, in a patient in Haiti suggests that the Caribbean and other regions of Latin America may be at risk for emergence of Mayaro virus epidemics. Alphavirus species EEEV and VEEV are endemic to the Americas and commonly cause severe neurological disease in animals and humans. VEEV epidemics have resulted in up to 100,000 human cases in Latin America, and an unexpected outbreak in Panama of human disease with Madariaga virus (MADV; also known as South American EEEV) was recently reported. Alphaviruses are demonstrably adaptable pathogens capable of rapidly expanding their ecological niche. For example, alphaviruses, including VEEV and CHIKV, can adapt to new vectors and hosts through mutations in viral envelope glycoproteins. Coupling this with changing patterns of vector distribution and abundance in response to climate change and increased vector-human contact following human encroachment into undeveloped areas renders alphaviruses emerging pathogens of high potential danger on a global scale. Unfortunately, no specific FDA-licensed antiviral therapies exist for any alphaviral species and no anti-alphaviral vaccines are licensed for human use.

Project 2 intends to use the existing platforms that have been developed at Southern Research (SR), OHSU (Daniel Streblow, (b) (3) (A), (b) (6)), University of North Carolina (b) (3) (A), (b) (6)), and University of Colorado (Tem (b) (3) (A), (b) (6) to identify and optimize drugs that directly target viral proteins of numerous alphaviruses, and perhaps viruses of other families. This includes nucleoside analog inhibitors of the viral RNA-dependent RNA polymerase (RdRp) and others that have been identified through the earlier work of the AD3C. Our intent is to discover molecules that are active against different viral proteins and, therefore, offer the opportunity for combination.

2. *Statement of the value of the project, including lay description of key technology objectives, innovation and advantages compared to competing products, technologies or services*

Currently, no antiviral drugs are licensed for use against any alphavirus. Importantly, given that alphaviruses are maintained in arthropod-dependent enzootic cycles vaccination will not eliminate these viruses. As such, the need for broadly effective anti-alphavirus drugs is great since alphaviruses will inevitably yet unpredictably emerge into human populations for the foreseeable future. The value of this project is the contribution of drugs to treat CHIKV and VEEV infection, and potentially many other alphavirus infections. Additional value of the project resides in the multidisciplinary approach of an experienced team of scientists with expertise in molecular biology, drug discovery, evaluation of antiviral activity, medicinal chemistry, animal models of viral pathogenesis, preclinical compound evaluation and, ultimately, the translation of our molecule discovery program into clinical development. The key technological objectives are the application of a seamless approach to rapid drug development. Lead molecules for the inhibition of CHIKV and VEEV replication have been identified that provide a technological starting point for product development. While other groups may pursue the discovery of lead molecules, such as those noted above, we have a distinct advantage of the availability of world class experts in viral biology and medicinal chemistry being an integral component of our application.

3. *Description of the goal(s) of the project, including one (or more) intermediate products, final product(s) or stage(s) of product development to be completed during the award period. (no specific product profile by licensing indication is needed)*

As noted above, the primary goal of the project is to develop drugs to treat alphavirus infection. We already have established most of the needed assays and have identified lead molecules that will be used to initiate mechanism of action studies with the opportunity for performing proof of principle early in this project. In addition, we have well-characterized RdRp inhibitors already display *in vivo* efficacy. Our goal is to identify additional hits and leads within a limited collection of nucleoside analogs and improve the spectrum of activity and efficacy of these lead products. Furthermore, mechanistic studies will be performed. A detailed research plan has been established with clear criteria for compound advancement. Using a **Go/No-Go** decision tree plan (see below under point 5), we attempt to finalize two to three molecules that will provide a choice for a preclinical development pathway leading to the selection of one or more clinical candidates, including the potential for combination.

4. *A detailed description of the qualitative and quantitative criteria and data elements to be used to assess the scientific merit of feasibility of proceeding to the next stage of development*

As a key aspect of their responsibilities our external Scientific Advisory Committee and the Executive Committee will work in a coordinated fashion to guarantee both prioritization and productivity of each project. As was learned in the current CETR program, ongoing decisions regarding continuation or discontinuation will be required.

On a more granular level, the research efforts and approaches leading to the selection of lead compounds are described in the individual project research strategy documents, as well as in the Assay and Medicinal Chemistry and Lead Development Core proposals. The goal is to identify lead compounds and eventually clinical candidates for the treatment of clinically important Alphavirus species, especially CHIKV and VEEV. In summary, the lead compounds identified will have demonstrated *in vivo* efficacy in two or more infectious animal models when administered orally. In addition, the compound's pharmacokinetic profile (half-life and bioavailability, distribution) would need to indicate that human doses of < 500 mg once or twice a day would be sufficient. Any compound progressing to this point would have had preliminary pharmacokinetic studies completed and have oral bioavailability in mice or rats of $\geq$30% and a minimum half-life of approximately 2 hours. Several additional studies would then be performed to advance the compound further. CNS exposure will also be determined. In addition, if the *in vitro* data warrant, these compounds would be tested across a panel of different viruses.

In parallel we will perform:

- Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors
- Compound stability as a dry powder and in solution at a range of pH's
- Examining the compound's effect on cytochrome P450's will be required as some of these compounds will be used in combination with other drugs
- Expanded PK studies including a second species (preferably dog, for which SR has the capability)
- Metabolite identification *in vivo*
- Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies
- A 7-day non-GLP toxicity study to determine a maximum tolerated dose, identify any toxicities and establish doses for the GLP toxicity study (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))

Most lead optimization programs produce multiple lead compounds, typically 2-4, and require an additional series of pre-GLP studies to select the compound with the best profile. The decision tree to select the drug candidate will be based upon the collection of properties of the compounds including efficacy, safety profile, physical properties (stability, solubility, crystal forms, etc.), pharmacokinetics, ease and cost of synthesis, dosage and formulation.

Monthly teleconference meetings of the Executive Committee will review ongoing data. At least yearly, the external Scientific Advisory Committee will meet to review the drug development plan. The NIH project officer for this grant will be invited to attend the yearly meetings to guide us regarding NIH partnerships that could facilitate product development. As we approach the identification of a clinical candidate, the external Scientific Advisory Committee will be able to assist the decision-making process.

From these targeted meetings, molecules will be selected for further development. Once a preclinical candidate is identified it would be tested in a fairly standard GLP program to collect the appropriate data for an IND filing. Initially, chemistry would involve synthetic route optimization to provide sufficient GMP API to support the GLP preclinical studies. Parallel with this would be further optimization to provide sufficient API for phase I/II clinical studies. Depending on the route of administration (oral delivery is preferred), formulation and stability studies would be conducted to optimize these routes (oral or IV, other options). Ideally, the compound will have the appropriate bioavailability and half-life for oral administration. Therefore, the Phase I studies will be conducted with a powder in a bottle formulation. The powder-in-bottle (PIB) approach will be used to accelerate development and introduction into Phase I clinical trials. This approach consists of extemporaneously compounding the active pharmaceutical ingredient (API) into a solution or a suspension in the clinic prior to oral administration. The development will consist of physico-chemical assessment, constitution fluid selection, weighing and dosing validation, and stability evaluation of API, before and after constitution with the fluid. This approach will provide a high degree of dosing flexibility during the initial phase of clinical trials. Additionally, the PIB approach reduced the time and API required for clinical development and supplies to < 2 months and < 100 gm, respectively.

A general GLP toxicity package would be developed. Full Ames and Mouse Lymphoma genotoxicity studies would be conducted. This would include acute oral and intravenous studies in rat and/or mouse and another species, typically dog for typically 28 days. The choice of species will depend in part upon the efficacy animal models. The length of the studies will be determined based upon the phase I/II clinical plan. A GLP safety pharmacology package would be generated that could include rat, dog (or primates depending on species selections above), Erwin studies and cardiovascular safety studies in 2 species and Perkinje Fiber electrophysiology. After IND filing, long term genotoxicity studies as well as longer term animal safety studies would be initiated. Additional funding for these studies would be required.

5. A description of the distinct stages/activities that are gates for Go/No Go decisions for advancement

From the set of experiments and capabilities outlined in the project proposal as well as in the Assay and Medicinal Chemistry and Lead Development Cores, compounds will be indentified with the following criteria:

1) *In vitro* antiviral activity and selectivity versus normal cells. Ranges for antiviral potency would be typically < 0.1 μM and > 20 μM versus normal cells.
2) Structure-activity relationships versus a primary endpoint indicating antiviral activity or mechanism
3) Solubility (≥10 μM), microsomal stability (>60% remaining at 30 minutes), measureable plasma free fractions (>1%) and an assessment of permeability (CaCo2).
4) No inhibition of cytochrome 3A4 or 2C9 at 10 μM.
5) A plasma half-life of >2 hr (mouse or rat) and >30% bioavailability.
6) Oral activity in a relevant mouse model of infection. No observable *in vivo* side effects. CNS exposure if relevant.
7) Understanding the metabolic pathways involved using both rodent, dog and human microsomes as well as *in vivo* in rodents and dogs prior to toxicology studies.
8) Tissue distribution
9) Synthetic feasibility determined (availability of starting materials, number of steps, time lines to produce 25-50 grams) and freedom to operate analysis completed

In summary, a preclinical compound would be orally active in a mouse model with dosing of ≤10 mg/kg and based upon the *in vitro* activity and pharmacokinetic profile the projected human dose would be <500 mg/day. A compound meeting these criteria from any of the projects would be designated a preclinical candidate and thus advance to a series of FDA regulated GLP studies required for IND submission.

The key milestones for the GLP studies and IND submission include:

1) GMP manufacturing of 500 -1000 grams of API to be used for GLP toxicity studies and formulation development. On average this can take approximately 6 months.
2) Identification of a second species for GLP toxicity studies. Typically dogs, but non-human primates may offer some advantages for certain indications. Once initiated the GLP toxicity studies will take about 6-9 months to complete the *in vivo* studies and generate the reports.
3) Preparation of the IND

6. *The performance specs/features the product should have in order to provide therapeutic benefit*

- Small molecule (MW <500), orally bioavailable
- No more than twice a day administration
- No more than 5-14 day treatment course
- Absence of bone marrow suppression or hepatic toxicity.
- Absence of development limiting adverse events (seizures in an animal model)
- Tissue distribution to target organs: lymphoid tissues, the reticulendothelial system, lung,

7. *A description and developmental status of the assays for the product release and characterization, including activity and efficacy (this may not be needed per se; depends on the stage of drug development)*

All of the assays for the performance of activity and efficacy determinations as well as initial characterization of the compounds exist within the confines of this application in either the participating institutions or in the Assay and Medicinal Chemistry and Lead Development Cores. These include in vitro screens of activity, mechanisms to develop resistant virus, monoclonal antibodies to assess drug distribution, and well characterized animal models. Furthermore, we have the resources of Southern Research to perform GLP clinical pharmacology and all preclinical toxicology required for an Investigational New Drug application. The certification, characterization, stability and release parameters of the GMP drug substance (API) will be conducted by an established GMP manufacturing company. The drug product development, stability and release will be contracted to an established Drug Product manufacturer.

7. *Discussion with the FDA, if any, relevant to the development activities of the candidate product.*

We envision involving the FDA on an informal basis (if allowed) in order to expedite drug development for treatment of Alphaviruses. This has worked most successfully for the PI (Richard Whitley) in studies performed by the NIAID Collaborative Antiviral Study Group. To be very specific, the NIAID CASG has had joint discussions with the Antiviral Division of the FDA involving unmet medical need and the proper clinical pathways to meet these needs. Toward this end, the Antiviral Branch of the FDA may have very specific issues that we will need to address in this application.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: Ph.D. Degree Year: 1997

Attach Biographical Sketch*: File Name: Streblow_U19_UAB_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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County: Washington
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E-Mail*: [redacted] (b) (3) (A), (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator   Other Project Role Category:

Degree Type: PHD,MS   Degree Year: 2001

Attach Biographical Sketch*: File Name: [redacted] (b) (3) (A), (b) (6)NIH_Biosketch_02-18_gb.pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator   Other Project Role Category:

Degree Type: PhD   Degree Year: 1996

Attach Biographical Sketch*: File Name: Biosketch_[redacted] (b) (3) (A), (b) (6)pdf
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name: (b) (3) (A), (b) Last Name*: (b) (3) (A), (b) (6) Suffix: Ph.D

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E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2004

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b).pdf
Attach Current & Pending Support: File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel		Streblow		Project Lead		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,223,206.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,223,206.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,223,206.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. Modified Total Direct Costs -1st 25,000 of 3 subawards	48.5	75,000.00	36,375.00
		Total Indirect Costs	**36,375.00**

Cognizant Federal Agency DHHS, Shon Turner, (214)-767-3261
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,259,581.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,259,581.00

L. Budget Justification* File Name:
Budget_Justification_Alpha_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel		Streblow		Project Lead		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,225,790.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,225,790.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,225,790.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,225,790.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,225,790.00

L. Budget Justification* File Name:
Budget_Justification_Alpha_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel	N.	Streblow	Ph.D	Project Lead		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	0.00

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	0.00

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,225,475.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,225,475.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,225,475.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,225,475.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,225,475.00

L. Budget Justification*

File Name:
Budget_Justification_Alpha_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel	N.	Streblow	Ph.D	Project Lead		(b) (6)	0.0	0.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,225,687.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,225,687.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,225,687.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,225,687.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,225,687.00

L. Budget Justification* File Name:
Budget_Justification_Alpha_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel	N.	Streblow	Ph.D	Project Lead		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,226,109.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,226,109.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**1,226,109.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**1,226,109.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	1,226,109.00

L. Budget Justification* File Name:
Budget_Justification_Alpha_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION PROJECT 2 ALPHAVIRUS

UAB has the prime CETR award; the Administrative Core will administer the subawards to the three project sites involved with Project 2. Please find the budget justification for those sites under their respective subaward budgets.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		6,126,267.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	6,126,267.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		6,126,267.00
Section H, Indirect Costs		36,375.00
Section I, Total Direct and Indirect Costs (G + H)		6,162,642.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		6,162,642.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel	N.	Streblow	Ph.D	Project Co-Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			21,801.00	7,630.00	29,431.00
2.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Co-Investigator	[redacted]	[redacted]			18,780.00	6,949.00	25,729.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **55,160.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			76,793.00	22,423.00	99,216.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Res Associate	[redacted]			45,133.00	13,540.00	58,673.00
2	Sr. Research Asst	[redacted]			36,391.00	13,648.00	50,039.00
1	Research Asst 2	[redacted]			12,521.00	6,636.00	19,157.00
1	Asst Staff Scient.	[redacted]			17,256.00	8,628.00	25,884.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**252,969.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**308,129.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Oregon Health & Science Univeristy
Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	82,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Sequencing services	1,871.00
9. Mouse Per Diems	4,000.00
Total Other Direct Costs	**89,871.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**400,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total Direct Costs (MTDC)	54.0	400,000.00	216,000.00
		Total Indirect Costs	**216,000.00**

Cognizant Federal Agency DHHD, Arif M. Karim, 214-767-36000

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**616,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	616,000.00

L. Budget Justification* File Name:

OHSU_U19_Budget_Justification_final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel	N.	Streblow	Ph.D	Project Co-Lead	(b) (6), (b) (4)	(b) (6)			22,455.00	7,859.00	30,314.00
2.	Dr.	[redacted]	[redacted]	(b) (6), (b) (3) (A)	Ph.D	Co-Investigator	[redacted]	[redacted]			19,344.00	7,157.00	26,501.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **56,815.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6)			79,096.00	23,097.00	102,193.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Res Associate	[redacted]			46,486.00	13,946.00	60,432.00
2	Sr. Research Asst	[redacted]			37,483.00	14,058.00	51,541.00
1	Research Asst 2	[redacted]			12,896.00	6,835.00	19,731.00
1	Asst Staff Scient.	[redacted]			17,774.00	8,887.00	26,661.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**260,558.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**317,373.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Oregon Health & Science Univeristy
Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	73,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Sequencing services	1,627.00
9. Mouse Per Diems	4,000.00
Total Other Direct Costs	**80,627.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**400,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total Direct Costs (MTDC)	54.0	400,000.00	216,000.00
		Total Indirect Costs	**216,000.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number): DHHD, Arif M. Karim, 214-767-36000

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**616,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	616,000.00

L. Budget Justification*

File Name:
OHSU_U19_Budget_Justification_final.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel	N.	Streblow	Ph.D	Project Co-Lead	(b) (6), (b) (4)	(b) (6)			23,128.00	8,095.00	31,223.00
2.	Dr.			(b) (3) (A), (b) (6)	Ph.D	Co-Investigator					19,924.00	7,372.00	27,296.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **58,519.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6)			81,469.00	23,789.00	105,258.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Res Associate				47,881.00	14,364.00	62,245.00
2	Sr. Research Asst				38,608.00	14,480.00	53,088.00
1	Research Asst 2				13,283.00	7,040.00	20,323.00
1	Asst Staff Scient.				18,307.00	9,154.00	27,461.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**268,375.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**326,894.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	64,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Sequencing services	1,106.00
9. Mouse Per Diems	4,000.00
Total Other Direct Costs	**71,106.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**400,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total Direct Costs (MTDC)	54.0	400,000.00	216,000.00
		Total Indirect Costs	**216,000.00**

Cognizant Federal Agency DHHD, Arif M. Karim, 214-767-36000
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**616,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	616,000.00

L. Budget Justification* File Name:
OHSU_U19_Budget_Justification_final.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel	N.	Streblow	Ph.D	Project Co-Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			23,822.00	8,338.00	32,160.00
2.	Dr.	[redacted]	[redacted]	[redacted] (b) (6), (b) (3) (A)	Ph.D	Co-Investigator	[redacted]	[redacted]			20,522.00	7,593.00	28,115.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **60,275.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			83,913.00	24,502.00	108,415.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Res Associate	[redacted]			49,318.00	14,795.00	64,113.00
2	Sr. Research Asst	[redacted]			39,766.00	14,915.00	54,681.00
1	Research Asst 2	[redacted]			13,681.00	7,251.00	20,932.00
1	Asst Staff Scient.	[redacted]			18,857.00	9,428.00	28,285.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**276,426.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**336,701.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	53,299.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Flow Cytometry Core Services	2,000.00
9. Mouse Per Diems	4,000.00
Total Other Direct Costs	**61,299.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**400,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total Direct Costs (MTDC)	54.0	400,000.00	216,000.00
		Total Indirect Costs	**216,000.00**

Cognizant Federal Agency DHHD, Arif M. Karim, 214-767-36000

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**616,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	616,000.00

L. Budget Justification* File Name:

OHSU_U19_Budget_Justification_final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Daniel	N.	Streblow	Ph.D	Project Co-Lead	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			24,537.00	8,588.00	33,125.00
2.	Dr.	[redacted]	[redacted]	(b) (3) (A), (b) (6) [redacted]	Ph.D	Co-Investigator	[redacted]	[redacted]			21,137.00	7,821.00	28,958.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **62,083.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6) [redacted]			86,431.00	25,237.00	111,668.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Res Associate	[redacted]			50,797.00	15,239.00	66,036.00
2	Sr. Research Asst	[redacted]			40,959.00	15,362.00	56,321.00
1	Research Asst 2	[redacted]			14,092.00	7,469.00	21,561.00
1	Asst Staff Scient.	[redacted]			19,422.00	9,711.00	29,133.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**284,719.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**346,802.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health & Science Univeristy

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Oregon Health & Science Univeristy
Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	43,198.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Flow Cytometry Core Services	2,000.00
9. Mouse Per Diems	4,000.00
Total Other Direct Costs	**51,198.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**400,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total Direct Costs (MTDC)	54.0	400,000.00	216,000.00
		Total Indirect Costs	**216,000.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) DHHD, Arif M. Karim, 214-767-36000

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**616,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	616,000.00

L. Budget Justification* File Name:
OHSU_U19_Budget_Justification_final.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – OREGON HEALTH & SCIENCE UNIVERSITY

PERSONNEL (Total Costs = $1,635,899)
Key Personnel:
Salaries and fringe benefits for year 1 are calculated at the rate projected for the period of October 1, 2018, through September 30, 2019, at Oregon Health & Science University (OHSU), and merit-based increases of 3% are calculated in each subsequent year. Fringe benefits are calculated based on the actual rate known for the individual. If actuals are unknown or personnel are to be determined, we utilize rates specified by the OHSU office of Central Financial Services for each salary level. Where investigator salaries exceed the NIH salary cap, only the amount of the cap will be requested here. The amount of salary above the cap will be covered by institutional funds.

Daniel N. Streblow, Ph.D. (Multiple-Principal Investigator/Contact PI, Years 1-5: (b) (6) effort, (b) (6) calendar months), is an Associate Professor in the Vaccine and Gene Therapy Institute (VGTI) at OHSU, with an adjunct position in the Division of Pathobiology and Immunology at the Oregon National Primate Research Center (ONPRC). Dr. Streblow will be the Multiple-PI for Project 3 with (b) (3) (A), (b) (6). Dr. Streblow will provide the oversight and organizational structure to ensure clear lines of authority and oversight of research activities, including those of all key personnel and technical staff. He will have responsibility for planning and maintaining communication with the other Projects and Cores and provide a direct line of communication with SR. Dr. Streblow will be responsible for helping to ensure compliance with all federal and NIH regulations as well as the safety and security of data, materials, facilities and resources of the program. Dr. Streblow has extensive experience with *in vitro* and *in vivo* animal models of alphavirus infection and disease as well as a background in pharmacology. Dr. Streblow will be responsible for data analysis and preparation of reports and publications derived from the project.

(b) (3) (A), (b) (6) Ph.D., Co-Investigator (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) will provide the oversight and organizational structure to ensure proper coordination and efficient execution of research activities in his lab, including those of key personnel, technical staff, and fee-for-service entities. (b) (3) (A), (b) (6) will hold the primary role in promoting scientific direction and interactions, ensuring research progress, and facilitating communications between research personnel, institutions, and the NIH. (b) (3) (A), (b) (6) will be responsible for maintaining compliance with all federal and NIH regulations as well as the safety and security of data, and managing materials, facilities and resources of the program. He has also conceived of, executed, and analyzed results from multi-factor experiments involving generation of large data sets such as *in vitro* high throughput screens and genome-wise transcriptomic profiling and is able to coordinate and troubleshoot all activities related to such undertakings. (b) (3) (A), (b) (6) regularly employs multiple *in vitro* and animal models of immunity, vaccination, and pathogenesis that can be implemented for the proposed studies.

Non-Key Personnel:
(b) (3) (A), (b) (6), Ph.D., Postdoctoral Scholar (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a postdoctoral scientist in the (b) (3) (A), (b) (6). (b) (3) (A), (b) (6) was trained in viral pathogenesis and immunology, and (b) (3) (A), has a strong background in immunology. (b) (3) (A), (b) (6) has provided initial characterization of the SR HTS hit compounds and their analogs. (b) (3) (A), (b) will devote much of her time to characterizing the lead molecules and will perform experiments to test the compounds in alphavirus infected animals. (b) (3) (A), (b) (6) previous work experience makes her an important addition to the group for completion of the proposed experiments.

(b) (3) (A), (b) (6), B.S., Senior Research Assistant (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a technician in the (b) (3) (A), (b) (6). He will be responsible for producing CHIKV and VEEV titered stocks for infection studies, performing dose titration experiments during medicinal chemistry phase, processing of animal samples for quantification of viral loads and assistance where necessary with other analyses. He will also be involved in producing recombinant alphaviruses containing resistance mutations on a wild type infectious clone backbone.

(b) (3) (A), (b) (6), Research Associate (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is an experienced member of the (b) (3) (A), (b) (6) with expertise in molecular diagnostics for CHIKV and VEEV. (b) (3) (A), (b) (3) (A), (b) (6) will aid in the generation of resistance phenotypes and molecular characterization by sequencing. (b) (3) (A), (b) (6) will also aid (b) (3) (A), (b) (6) in compound protection experiments and studies to understand the molecular mechanisms involved in compound mode of action.

(b) (3) (A), (b) (6), Senior Research Assistant (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a researcher and operational manager in the (b) (3) (A), (b) (6) (b) (3) (A), (b) is responsible for facilitating numerous aspects of laboratory function, including procuring, maintaining, organizing, and curating supplies and reagents, cell line maintenance, and sample storage. (b) (3) (A), (b) will be crucially involved in cell culture, cellular analysis, and molecular analysis (cytokine, RNA, protein). (b) (3) (A), (b) is also responsible for maintaining databases of biological samples and reagents and maximizing research efficiency.

(b) (3) (A), (b) (6) Ph.D., Postdoctoral Scholar (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a postdoctoral scientist in the (b) (3) (A), (b) (6) (b) (3) (A), (b) possesses expertise in the area of innate immune signaling pathways and viral replication. For this she is skilled at employing a diverse array of *in vitro* techniques including transgenic cell line construction, CRISPR/Cas9-mediated genome editing, and transcriptomics. (b) (3) (A), (b) is also highly skilled at confocal fluorescence microscopy and cellular imaging techniques. (b) (3) (A), (b) will primarily be responsible for performing and analyzing all IFA experiments, generating RNA samples for RNAseq as well as devising, directing, and troubleshooting all *in vitro* mechanism of action experiments in coordination with (b) (3) (A), (b) (6) and (b) (3) (A), l.

(b) (3) (A), (b) (6), Ph.D., Postdoctoral Scholar (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a postdoctoral scientist in the (b) (3) (A), (b) (6). He possesses a wide range of skills involving *in vivo*, *in vitro*, and *ex vivo* examination of inflammatory, innate, and adaptive immune responses, molecular and immunological data collection and analysis, and characterization of stimuli-induced phenotypes. He will primarily be responsible for executing and interpreting all secondary validation and *in vitro* phenotypic assays. He will also be involved in facilitating animal experiments in coordination with (b) (3) (A), (b) (6) and be centrally involved in statistical data analysis and experimental design.

(b) (3) (A), (b) (6)l, Ph.D., Assistant Staff Scientist (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a senior researcher in the (b) (3) (A), (b) (6). He possesses expertise in the areas of transgenic cell line construction using multiple technical platforms including lentivectors and transposons and has developed novel methods for optimal in-house application of these methods. He is also highly skilled at CRISPR/Cas9-mediated genome editing and characterization of innate immune signaling pathways and phenotypes. In coordination with (b) (3) (A), (b) (6) he will primarily be responsible for constructing optimal reporter cell lines and generating master cell banks.

(b) (3) (A), (b) (6), M.S., Research Assistant 2 (Years 1-5, (b) (6) effort (b) (6) calendar months). (b) (3) (A), (b) (6) is a researcher in the (b) (3) (A), (b) (6). (b) (3) (A), (b) possesses an array of talents including molecular cloning, lentivector construction and utilization, CRISPR/Cas9-driven cell line construction, quantitative transcriptional and protein analysis, mouse husbandry and experimentation, and high throughput assays. Under the direction of (b) (3) (A),. (b) (3) (A), (b) (6) (b) (3) (A), (b) will be responsible for employing these skills to provide assistance with animal work, processing biomaterials for analysis, construction of cell lines, and molecular biological experiments.

MATERIALS AND SUPPLIES (Total Costs = $315,497)

Tissue Culture Supplies: These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes. ($25,000 in Year 1; $20,000 in Year 2; $20,000 in Year 3; $17,000 in Year 4; and $14,000 in Year 5)

Molecular Biology Reagents: These will be required for all molecular biological and biochemical experimentation, including qPCR (for viral quantification), immunoblotting (to monitor antibody specificity), immunofluorescence, and viral replication monitoring/evaluation. This includes enzymes (e.g., Taq polymerase) and chemicals (e.g., SDS, TRIS base, agarose, TEMED, Tween, BSA, formalin, ethanol, DMSO), agar, and LB broth. ($22,000 in Year 1; $20,000 in Year 2; $14,000 in Year 3; $9,299 in Year 4; and $5,198 in Year 5)

Antibodies: Antibodies will be required for flow cytometry to identify changes in host lymphocyte phenotype and activation status. ($2,000/yr in Years 1-5)

LiqN$_2$ & CO$_2$: CO$_2$ will be required for all tissue culture. Liquid nitrogen will be required for cryopreservation of all primary cells and cell lines. ($2,000/yr in Years 1-5)

Plasticware: Disposable plasticware will be required for cell and virus culture, virus titration and virus isolation, and molecular biological work. This includes tissue culture dishes of diverse sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. ($25,000 in Year 1; $23,000 in Year 2; $20,000 in Year 3; $17,000 in Year 4; and $14,000 in Year 5)

Mouse Sample Collection/Drug Administration Supplies: Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs will be required for obtaining blood samples and tissues. ($1,000/yr in Years 1-5)

Mouse purchase fees**:** For the testing of compounds in our mouse models we will require up to 250 mice/yr x $20 each = $5,000. We are requesting $5,000 in each year of the project (Years 1-5). Total = $25,000

OTHER EXPENSES (Total Costs = $28,604)

Flow Cytometry Core Services: T cell antigen reactivity and innate/adaptive immune cell phenotypic characterization and activation will be assessed using flow cytometry in the VGTI core facility, which employs a fee-for-service operational model. ($2,000 in Years 4 and 5)

Sequencing Services: We are requesting $1,871 in Year 1, $1,627 in Year 2, and $1,106 in Year 3 for sequencing services and supplies to characterize virus resistance mutants.

Mouse per diems: Per diem costs are $1.20/day x 50 cages (5 animals per cage) x 67 days = $4,000 per year. We are requesting $4,000 in per diem fees for each year of the project. Total = $20,000

PUBLICATIONS (Total Costs = $10,000)

Results will be published in peer-reviewed journals as they are satisfactorily completed. ($2,000/yr in Years 1-5)

TRAVEL (Total Costs = $10,000)

Travel costs have been allocated for one scientific meeting per year for Drs. Streblow, and (b) (6), (b) (3) (A). This will be necessary to communicate results obtained from the proposed studies and to stay current on related outside research activities and protocols. Funds in the amount of $2,000 annually for Years 1-5 are requested for round-trip airfare, per diems (meals and lodging), meeting registration and miscellaneous travel expenses (parking, ground transportation) based on up to four nights of lodging and five days of per diem. The daily maximum amount for meals and lodging may vary, based on location in high-cost areas.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		292,852.00
Section B, Other Personnel		1,343,047.00
Total Number Other Personnel	40	
Total Salary, Wages and Fringe Benefits (A+B)		1,635,899.00
Section C, Equipment		0.00
Section D, Travel		10,000.00
1. Domestic	10,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		354,101.00
1. Materials and Supplies	315,497.00	
2. Publication Costs	10,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	8,604.00	
9. Other 2	20,000.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		2,000,000.00
Section H, Indirect Costs		1,080,000.00
Section I, Total Direct and Indirect Costs (G + H)		3,080,000.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		3,080,000.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			17,642.00	4,696.00	22,338.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **22,338.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			40,278.00	6,860.00	47,138.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Technical staff	[redacted]			42,468.00	14,293.00	56,761.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**103,899.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,237.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: The University of North Carolina at Chapel Hill
Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	2,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	56,263.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Maintenance contracts & repairs	4,500.00
9. Histology	5,000.00
Total Other Direct Costs	**67,763.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MTDC	55.5	200,000.00	111,000.00
		Total Indirect Costs	**111,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Division of Cost Allocation Darryl Mayes 202-401-2808

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**311,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	311,000.00

L. Budget Justification* File Name: [redacted] (b) (3) (A), (b) (6) pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			17,642.00	4,696.00	22,338.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **22,338.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			40,278.00	6,860.00	47,138.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Technical staff	[redacted]			42,468.00	14,293.00	56,761.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**103,899.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,237.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	2,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	56,263.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Maintenance contracts & repairs	4,500.00
9. Histology	5,000.00
Total Other Direct Costs	**67,763.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MTDC	55.5	200,000.00	111,000.00
		Total Indirect Costs	**111,000.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**311,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	311,000.00

L. Budget Justification* File Name: [redacted] (b) (3) (A), (b) (6) pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			17,642.00	4,696.00	22,338.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **22,338.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			40,278.00	6,860.00	47,138.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Technical staff	[redacted]			42,468.00	14,293.00	56,761.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**103,899.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,237.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	2,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: The University of North Carolina at Chapel Hill
Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	56,263.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Maintenance contracts & repairs	4,500.00
9. Histology	5,000.00
Total Other Direct Costs	**67,763.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MTDC	55.5	200,000.00	111,000.00
		Total Indirect Costs	**111,000.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**311,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	311,000.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			17,642.00	4,696.00	22,338.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **22,338.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			40,278.00	6,860.00	47,138.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Technical staff	[redacted]			42,468.00	14,293.00	56,761.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**103,899.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,237.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	2,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	56,263.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Maintenance contracts & repairs	4,500.00
9. Histology	5,000.00
Total Other Direct Costs	**67,763.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MTDC	55.5	200,000.00	111,000.00
		Total Indirect Costs	**111,000.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**311,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	311,000.00

L. Budget Justification* File Name: [redacted] (b) (3) (A), (b) (6) pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted] (b) (3) (A), (b) (6)			Ph.D	Project Co-investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			17,642.00	4,696.00	22,338.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **22,338.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			40,278.00	6,860.00	47,138.00
	Graduate Students	[redacted]					
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
2	Technical staff	[redacted]			42,468.00	14,293.00	56,761.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**103,899.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,237.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	2,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	56,263.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Maintenance contracts & repairs	4,500.00
9. Histology	5,000.00
Total Other Direct Costs	**67,763.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. MTDC	55.5	200,000.00	111,000.00
		Total Indirect Costs	**111,000.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**311,000.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	311,000.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6) pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel:

(b) (3) (A), (b) (6) **Ph.D., Co-Investigator** (b) (6) Calendar Months, (b) (6) effort). (b) (3) (A), (b) (6) has expertise in viral pathogenesis and viral immunology, and has worked with models of alphavirus pathogenesis, including CHIKV and VEEV for (b) (3) (A), (b) (6). He will be responsible for the overseeing the testing of candidate therapeutics for their ability to protect mice from acute CHIKV induced arthritis or VEE-induced viral encephalitis. He will work in close collaboration with (b) (3) (A), (b) (6) and Streblow, as well as the other research project leaders to set priorities for which drugs should be tested within the CHIKV or VEE models and to identify promising candidates that show promising in vivo potential so that those candidates can be taken forward for further optimization.

(b) (3) (A), (b) (6)**, Ph.D. Postdoctoral Fellow** ((b) (6) Calendar Months, (b) (6) effort). (b) (3) (A), (b) (6) has (b) (3) years of experience working with both CHIKV and VEE in mouse models and will work in close coordination with (b) (3) (b) (3) (A), (b) (6) to test candidate therapies for their ability to protection from CHIKV or VEE-induced disease. He will administer therapeutics, perform CHIKV and VEE infections, and will monitor infected animals for disease signs and collect tissues to assess viral loads and virus-induced pathology. He also directs the day to day operations of the BSL-3 facility where all work with CHIKV and VEE is conducted and will oversee the proper training and compliance of all individuals working within the BSL-3 facility.

(b) (3) (A), (b) (6)**, Ph.D. Research Associate** (b) (6) Calendar Months, (b) (6) effort). (b) (3) (A), (b) (6) has approximately (b) (3) years of experience working with VEE and CHIKV infected mice under BSL-3 conditions. (b) (3) (A), (b) will be responsible for coordinating in vivo mouse studies and will be involved in the administration of candidate therapies and viral challenge studies. (b) (3) will also assist in collection of data to assess the impact of therapeutics on viral loads, virus-induced disease, and virus induced pathology within joint (CHIKV) and the central nervous system (VEE).

(b) (3) (A), (b) (6)**, Research Technician** (b) (6) Calendar Months, (b) (6) effort). (b) (3) (A), (b) (6) performs a wide range of duties that support the viral challenge studies within the BSL-3 laboratory. (b) (3) (A), (b) has extensive experience in cell culture and molecular virology and will be responsible for assisting in the production of VEE and CHIKV virus stocks and in conduct plaque assays to assess viral loads in CHIKV or VEE infected mice. (b) (3) (A), (b) will also process tissues to assess virus-induced pathology and will perform real time PCR analysis to evaluate viral RNA loads in virally infected tissues in the presence or absence of candidate therapeutics.

Fringe Benefits: Faculty/Staff: 23.293% Social Security and Retirement; $5869/FTE Health Insurance. Postdoctoral fellow benefits are calculated at 8.99% of salary and $4318/FTE for health insurance.

SUPPLIES ($56,263):

The evaluation of candidate therapies against either CHIKV or VEE requires the assessment of viral loads, evaluation of inflammatory cell infiltration and pathology within these tissues. Therefore funds are requested to cover the costs of tissue culture consumables (plastic ware, media, serum) required for the assessment of viral loads within the joints or CNS. We will also need to generate viral stocks, as well as generate infectious clones containing potential escape mutants and funds are requested to cover the costs of the molecular biology supplies needed for those purposes. We are also requesting funds to cover the cost of purchasing adult C57Bl/6 mice, which will be used for testing candidate therapies against both CHIKV and VEE, as well as funds to cover the cost of supplies needed to house these animals within our BSL-3 laboratory. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective gears, such as gloves, tyvek suits, and PAPRs.

TRAVEL ($6,000):

Funds are requested for the Project Leader, and 2 lab members to attend 1 scientific meeting to present findings and interact with other scientists in the field and to attend programmatic meetings.

OTHER EXPENSES ($11,500):

Equipment service contracts ($4,500): Several instruments in the (b) (3) (A), (b) (6) that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology Costs ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Publication Costs ($2,000) Funds are requested to cover the cost of publishing scientific results from this project.

Federal F&A Cost Rate: In accordance to an agreement between DHHS and UNC dated November 23, 2016, the indirect cost rate is 55.5% of modified total direct costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		111,690.00
Section B, Other Personnel		519,495.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		631,185.00
Section C, Equipment		0.00
Section D, Travel		30,000.00
1. Domestic	20,000.00	
2. Foreign	10,000.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		338,815.00
1. Materials and Supplies	281,315.00	
2. Publication Costs	10,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	22,500.00	
9. Other 2	25,000.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,000,000.00
Section H, Indirect Costs		555,000.00
Section I, Total Direct and Indirect Costs (G + H)		1,555,000.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		1,555,000.00

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]		(b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			11,634.00	2,913.00	14,547.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **14,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6) [redacted]			23,742.00	6,038.00	29,780.00
1	Graduate Students	[redacted]			30,000.00	0.00	30,000.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Technician	[redacted]			21,580.00	8,090.00	29,670.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**89,450.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**103,997.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1. 1. Refrigerator	5,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**5,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		11,311.00
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**11,311.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	48,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	22,500.00
Total Other Direct Costs	**73,000.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**196,308.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total DIrect Costs	55.5	179,997.00	99,898.00
		Total Indirect Costs	**99,898.00**

Cognizant Federal Agency DHHS, Arif Karim 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**296,206.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	296,206.00

L. Budget Justification* File Name:
[redacted](b) (3) (A), (b) (6)pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			11,983.00	3,001.00	14,984.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **14,984.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			24,455.00	6,219.00	30,674.00
1	Graduate Students	[redacted]			30,000.00	0.00	30,000.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Technician	[redacted]			22,228.00	8,333.00	30,561.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**91,235.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**106,219.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		12,329.00
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**12,329.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	48,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	24,500.00
Total Other Direct Costs	**75,000.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**196,548.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total DIrect Costs	55.5	184,219.00	102,242.00
		Total Indirect Costs	**102,242.00**

Cognizant Federal Agency DHHS, Arif Karim 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**298,790.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	298,790.00

L. Budget Justification* File Name:
[redacted] (b) (3) (A), (b) (6) pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted]	[redacted]	[redacted] (b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator	(b) (6), (b) (4) [redacted]	(b) (6) [redacted]			12,342.00	3,090.00	15,432.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **15,432.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6) [redacted]			25,188.00	6,405.00	31,593.00
1	Graduate Students	[redacted]			30,000.00	0.00	30,000.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Technician	[redacted]			22,895.00	8,583.00	31,478.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**93,071.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**108,503.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	13,439.00
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**13,439.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	33,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	36,300.00
Total Other Direct Costs	**71,800.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**196,742.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total DIrect Costs	55.5	183,303.00	101,733.00
		Total Indirect Costs	**101,733.00**

Cognizant Federal Agency DHHS, Arif Karim 415-437-7820

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**298,475.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	298,475.00

L. Budget Justification* File Name:

(b) (3) (A), (b) (6)pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted] (b) (3) (A), (b) (6)			Ph.D	Project Co-Investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			12,713.00	3,183.00	15,896.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **15,896.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			25,944.00	6,598.00	32,542.00
1	Graduate Students				30,000.00	0.00	30,000.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician				23,582.00	8,841.00	32,423.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**94,965.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**110,861.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	14,649.00
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**14,649.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	33,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	33,300.00
Total Other Direct Costs	**68,800.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**197,310.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total DIrect Costs	55.5	182,661.00	101,377.00
		Total Indirect Costs	**101,377.00**

Cognizant Federal Agency DHHS, Arif Karim 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**298,687.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	298,687.00

L. Budget Justification* File Name:
[redacted] (b) (3) (A), (b) (6) pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	[redacted] (b) (6), (b) (3) (A)			Ph.D	Project Co-Investigator	[redacted] (b) (6), (b) (4)	[redacted] (b) (6)			13,094.00	3,279.00	16,373.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **16,373.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted] (b) (6)			26,722.00	6,795.00	33,517.00
1	Graduate Students	[redacted]			30,000.00	0.00	30,000.00
	Undergraduate Students	[redacted]					
	Secretarial/Clerical	[redacted]					
1	Technician	[redacted]			24,289.00	9,106.00	33,395.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**96,912.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**113,285.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	15,967.00
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**15,967.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period:** 5

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	33,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	30,300.00
Total Other Direct Costs	**65,800.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**198,052.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1. 1. Modified Total DIrect Costs	55.5	182,085.00	101,057.00
		Total Indirect Costs	**101,057.00**

Cognizant Federal Agency DHHS, Arif Karim 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**299,109.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	299,109.00

L. Budget Justification* File Name:
[redacted] (b) (3) (A), (b) (6).pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

[redacted] (b) (3) (A), (b) (6)

PERSONNEL ($103,997):

[redacted] (b) (3) (A), (b) (6) **Ph.D., Principal Investigator.** Associate Professor of Immunology and Microbiology at [redacted] (b) (3) (A), (b) (6). [redacted] (b) (3) (A), (b) (6) will devote [redacted] (b) (6) calendar months to this project and receive [redacted] (b) (6) calendar months of salary support. [redacted] (b) (3) (A), (b) (6) has extensive experience and expertise in alphavirus pathogenesis, host responses to virus infection, and mouse models of infectious diseases. [redacted] (b) (3) (A), (b) (6) will be responsible for the design and oversight of all investigations occurring in his laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

[redacted] (b) (3) (A), (b) (6)**, Professional Research Assistant.** [redacted] (b) (3) (A), (b) (6) will devote [redacted] (b) (6) calendar months of time to this project and derive [redacted] (b) (6) calendar months of salary from it. [redacted] (b) (3) (A), (b) (6) will assist with the general management of this project. He will assist with management of the mouse colony, tissue culture experiments, generation of stocks of infectious viruses for assays, RNA isolations, and PCR analysis. [redacted] (b) (3) (A), (b) (6) also will be responsible for general lab management, inventory, and reagent/supply orders.

TDB, Graduate Student. A graduate student will be recruited for this project. This person will devote [redacted] (b) (6) calendar months of time to this project and derive [redacted] (b) (6) calendar months of salary from it. This person will be responsible for performing tissue culture experiments to define mechanisms of action of anti-viral inhibitors. This person also will perform inoculations of mice, administer therapeutics, perform animal necropsies, and process tissues for quantification of viral burdens and for the assessment of tissue pathology.

TBD, Postdoctoral Researcher. A postdoctoral researcher will be recruited for this project. This person will devote [redacted] (b) (6) calendar months of time to this project and derive [redacted] (b) (6) calendar months salary from it. This person will perform tissue culture experiments to define mechanisms of resistance to antiviral compounds and animal infection experiments to evaluate combination anti-viral plus anti-inflammatory therapeutics. This person also will assist with data analysis, data interpretation, and manuscript preparation.

EQUIPMENT ($5,000):

We request funds to purchase one -80°C freezer in FY1. We will cost-share this equipment with another project and thus are requesting only 50% of the funds to come from this grant. This will be used to store all materials (viral samples, RNA, serum samples) related to this project.

SUPPLIES (Y1: $48,500; Y2: $48,500; Y3: 33,500; Y4: $33,500, Y5: 33,500):

Tissue culture. The funds requested will be used for media preparation, serum, antibiotics, and plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles).

Molecular Biology Reagents. Funds are requested to cover the costs of consumables such as Trizol, RNA isolation kits, reverse-transcriptase enzyme, and PCR reagents. These reagents will be used for quantification of CHIKV RNA in tissues and cells via quantitative real-time RT-PCR. We also request funds for antibodies and consumables used to evaluate viral titers by plaque assays or focus-forming assays In addition, the performance of these studies requires stocks of infectious CHIKV, VEEV, and other alphaviruses generated from cDNA clones. Therefore, we request funds for restriction enzymes, plasmid DNA purification kits, bacterial culture supplies, DNA/RNA electrophoresis, and *in vitro* transcription kits.

General. We request funds to cover the costs associated with the generation of fixed frozen tissue sections ($5 per section), paraffin-embedded tissue sections ($6.50 per section), and staining of tissue sections with hematoxylin and eosin or other special stains for the evaluation of histopathological changes ($3.25 per section). This amount also includes funds for liquid N2/CO2 bottled gas and BSL3 biosafety laboratory operation (personal protective equipment and BSL3 waste disposal).

OTHER EXPENSES:

Animals (Y1: $22,500; Y2: $24,500; Y3: 36,300; Y4: $33,300; Y5: $30,300):

Animal purchase. The acute and chronic CHIKV infection model and the VEEV model utilizes WT C57BL/6 mice. Therefore, we request funds to cover costs associated with the purchase WT C57BL/6 mice ($22/mouse; JAX mice). CHIKV and VEEV infection studies also may use genetically altered mice that display enhanced sensitivity to infection.

Animal breeding. Funds are requested to maintain breeders of WT and genetically altered strains of C57BL/6 mice in the AMC mouse facility ($0.92 per cage per day).

Animal housing. Funds are requested for cage charges for all mice immediately before and after infection with VEEV or CHIKV, BSL2 and BSL3 pathogens depending on the strain used. All infection experiments will take place in the ABSL2 or ABSL3, which has higher per diems ($1.11 per cage).

Travel ($3,000):

Travel is requested for (b) (3) (A), (b) (6) and the graduate student working on the project to attend one meeting per year for presentation of scientific findings.

Publication Costs ($2,000):

Funds are budgeted for publication costs.

Tuition Remission ($11,311):

Tuition remission is requested for (b) (3) (A), (b) (6), graduate student.

Specialized Costs associated with BSL3. In comparison to standard BSL2 work, additional costs are incurred for BSL3 and ABSL3 work. These include user fees for the BSL3 that are paid to the institution to offset the cost of the facility, the purchase and utilization of personal protection equipment (PPE) for every experiment, biosafety waste management, and higher animal facility cost. **Given the extensive animal experiments conducted under ABSL3 conditions with pathogenic strains of CHIKV, our costs are necessarily greater than standard BSL2 work**.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		77,232.00
Section B, Other Personnel		465,633.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		542,865.00
Section C, Equipment		5,000.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		67,695.00
1. Tuition/Fees/Health Insurance	67,695.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		354,400.00
1. Materials and Supplies	197,500.00	
2. Publication Costs	10,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	146,900.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		984,960.00
Section H, Indirect Costs		506,307.00
Section I, Total Direct and Indirect Costs (G + H)		1,491,267.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		1,491,267.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Project2_NovelAlphavirusAntivirals_v11_specific_aims_page.pdf
3. Research Strategy*	Project2_NovelAlphavirusAntivirals_research_strategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	VA_Alphavirus.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	UAB_U19_Resource__Sharing__Plan.pdf
11. Authentication of Key Biological and/or Chemical Resources	UAB_U19_Authentication_key_resources_gb.pdf
Appendix	
12. Appendix	

SPECIFIC AIMS

The goal of this project is the continued development of novel antiviral drugs against emerging alphaviruses. Alphaviruses are a clade of arthropod-transmitted RNA viruses comprised of multiple NIAID Category B and C priority pathogens including Venezuelan equine encephalitis (VEEV) and chikungunya (CHIKV) viruses, as well as other re-emerging viruses such as Eastern equine encephalitis (EEEV), Mayaro (MAYV), o'nyong-nyong (ONNV), and Ross River (RRV) viruses. The encephalitic alphaviruses, such as EEEV and VEEV, primarily present with neurological disease that can result in high mortality rates in hosts with weakened or immature immune systems, including young and aged populations. Arthritogenic alphaviruses, including CHIKV, MAYV, and RRV, are associated with incapacitating acute and chronic arthralgia, arthritis, and tenosynovitis that can result in long-term disability, and large epidemics with severe economic consequences. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents.

In collaboration with Southern Research (SR), we performed high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. Furthermore, we generated resistant mutants against two highly active compounds and, in doing so, identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as potential antiviral targets. In addition to this work, collaboration with the Emory Institute for Drug Development (EIDD) identified novel nucleosides, which target RNA-dependent RNA polymerases, with antiviral activity against alphavirus infection *in vitro* and *in vivo*. Thus, we have identified potent antiviral compounds against three distinct molecular targets of alphavirus replication.

In this CETR application, we plan to (i) further chemically optimize the lead compounds for antiviral potency, efficacy, and drug-like properties, (ii) define the phylogenetic breadth of antiviral activity, mechanisms of action, and synergism of lead compounds, and (iii) evaluate antiviral activity with single agents and in combination with other leads identified in this project. In addition, we will explore combinations of these antiviral compounds with anti-inflammatory therapies against alphavirus infection in well-characterized pre-clinical animal models of acute and chronic alphavirus disease. To develop novel inhibitors that block alphavirus replication, prevent the emergence of resistance viruses, and reduce disease severity, we propose the following Specific Aims:

Specific Aim 1. To further optimize the inhibitors of alphavirus replication to improve potency and bioavailability. Our previous work resulted in the identification of four chemically distinct leads (**SRI-33001, SRI-33394, SRI-34626,** and **SRI-34963**). These leads display potent antiviral activity against diverse alphaviruses with low cytotoxicity. Furthermore, we identified novel nucleoside inhibitors of alphavirus replication, including the lead compound **EIDD-1931** and prodrug (**EIDD-2801**), as well as an active 4'fluorouridine analog **EIDD-2749**. In this Aim, these lead compounds will be optimized by iterative medicinal chemistry, cell culture based antiviral and cytotoxicity assays, and *in vivo* pharmacokinetic studies to improve their efficacy, selectivity, solubility, and bioavailability. Optimized compounds will advance to mechanistic (SA2) and pivotal efficacy (SA3) studies.

Specific Aim 2. To determine the antiviral mechanisms of inhibitors of alphavirus replication. In this Aim, we will define the breadth of antiviral activity and cell type specificity, determine the molecular targets through resistance mapping and structural/computational analyses, and identify synergy profiles for active compounds. In preliminary studies, characterization of resistant mutants derived against two highly inhibitory compounds identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as likely molecular targets. To explore the activity of compounds against these specific viral targets, we have generated a cell line expressing nsP2 helicase and purified nsP2 protein for biochemical/structural assays. Furthermore, we have generated a 1.46 Å resolution crystal structure of the nsP3 macrodomain. We will use this new structural information for *in silico* computational modeling and empirical structural biology analysis to identify regions of the macrodomain targeted by small molecule inhibitors and their analogs. Finally, we will define the mechanisms of action for our anti-alphavirus compounds and structurally related active analogs that are yet uncharacterized. These studies will inform our compound selections for synergy studies in the context of combination therapy (SA3).

Specific Aim 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease. In this Aim, compounds with optimized antiviral and pharmacokinetic profiles will be evaluated using well-established pre-clinical murine models of alphavirus infection and disease. Given that selection for antiviral drug resistance can adversely impact therapeutic efficacy, and that combination therapies targeting two or more regions of the virus should generate a higher barrier to resistance, new therapeutic regimens will be tested that combine our small molecule inhibitors with novel nucleoside analogs that show robust *in vivo* efficacy. Lastly, since alphavirus disease is caused in part by virus-induced inflammatory immune pathology, we will evaluate the therapeutic potential of antiviral therapy combined with anti-inflammatory regimens to block both virus replication and virus-induced immune pathology.

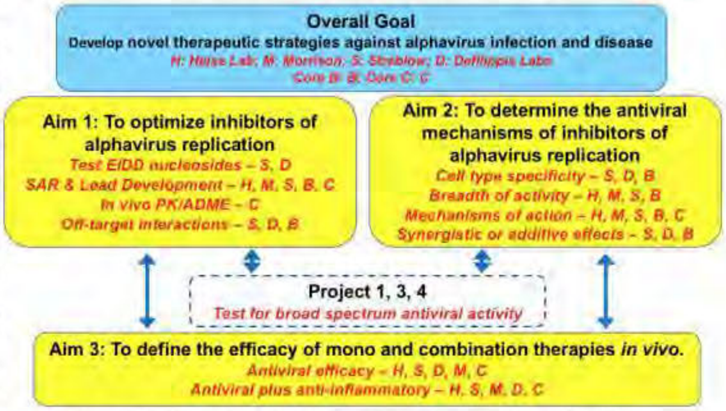
RESEARCH STRATEGY

A. SIGNIFICANCE. Mosquito-transmitted alphaviruses are re-emerging positive-sense RNA viruses that include multiple NIAID Category B and C Priority Pathogens including Venezuelan equine encephalitis (VEEV), western equine encephalitis (WEEV), eastern equine encephalitis (EEEV), and chikungunya (CHIKV) viruses. Encephalitic alphaviruses can cause high mortality rates in hosts with weakened or immature immune systems including the young and aged populations[1, 2 3], and pose a serious threat to public health during outbreaks. The arthritogenic alphaviruses, including CHIKV, cause incapacitating acute and chronic musculoskeletal inflammatory disease that can result in long-term disability especially in older adults. Furthermore, older adults and young children are susceptible to life-threatening CHIKV disease. CHIKV outbreaks have historically occurred in sub-Saharan Africa and Southeast Asia[4]; however, an outbreak in India and Southeast Asia between 2005 until 2015[5] was unprecedented in scale, affecting millions of people. In 2013, CHIKV was introduced into the Western Hemisphere and thus far, has resulted in approximately 2 million infections throughout the Americas.[6] CHIKV also has adapted to *Aedes albopictus* mosquitoes[7], which further increases the transmission risk of CHIKV in more temperate climates such as the United States and Europe.

There are currently no FDA-licensed vaccines or therapeutics to prevent or treat alphavirus infections. Thus, there is a critical unmet need for the development of therapeutics against re-emerging alphaviruses. *The proposed research is highly significant as it is uniquely positioned to identify novel therapeutic targets and the development of new antiviral therapies against multiple alphaviruses, including the high consequence pathogens CHIKV and VEEV.* Our multidisciplinary team of virologists, medicinal chemists, structural and computational biologists, along with leaders in drug discovery, will optimize our lead antiviral molecules and obtain pre-clinical data that will ultimately move these new antiviral molecules toward testing in humans.

B. INNOVATION. The proposed research is highly innovative in part due to the complete absence of any therapeutics against alphavirus infection. Innovation also arises from the fact that we have developed novel and potent lead compounds against non-overlapping targets in the viral replicase [nsP4-RdRp (**EIDD-1931**), nsP2-helicase (**SRI-36129**), and nsP3-macrodomain (**SRI-36498**)], which should allow us to develop highly efficacious combination therapies less likely to select for drug resistance. Innovation also is derived from our generation of potent leads with inhibitory activity against multiple alphaviruses, highlighting the potential for developing pan-alphavirus therapies. Furthermore, several of our lead compounds exhibit inhibitory activity across multiple virus families (alphaviruses, coronaviruses, flaviviruses, and influenza virus), raising the possibility that our highly collaborative CETR research team can develop broadly acting antivirals against a number of viral pathogens.

C.1. SCIENTIFIC PREMISE. The premise of the proposed research is based on scientific precedent, as basic and translational research has generated effective clinical treatments against viral infections such as HIV and HCV. Furthermore, our previous work with CHIKV and VEEV identified several lead compounds with drug-like properties and inhibitory activity against multiple alphaviruses and other virus families. For example, **EIDD-1931** has potent inhibitory activity against VEEV[8] as well as other alphaviruses, influenza virus, coronaviruses, and flaviviruses. Our lead molecules will be chemically optimized for drug-like properties and broad antiviral activity and tested for *in vitro* and *in vivo* efficacy. A clear progression plan will ensure that we reach our proposed milestones.


Overall Goal
Develop novel therapeutic strategies against alphavirus infection and disease
H: Heise Lab; M: Morrison; S: Streblow; D: DeFilippis Labs
Core B: B; Core C: C
Aim 1: To optimize inhibitors of alphavirus replication
Test EIDD nucleosides – S, D
SAR & Lead Development – H, M, S, B, C
In vivo PK/ADME – C
Off-target interactions – S, D, B
Aim 2: To determine the antiviral mechanisms of inhibitors of alphavirus replication
Cell type specificity – S, D, B
Breadth of activity – H, M, S, B
Mechanisms of action – H, M, S, B, C
Synergistic or additive effects – S, D, B
Project 1, 3, 4
Test for broad spectrum antiviral activity
Aim 3: To define the efficacy of mono and combination therapies in vivo.
Antiviral efficacy – H, S, D, M, C
Antiviral plus anti-inflammatory – H, S, M, D, C

C.2. EXPERIMENTAL RIGOR AND CONSIDERATION OF RELEVANT BIOLOGICAL VARIABLES. To improve precision, all *in vitro* experiments will be performed using multiple technical and biological replicates. Proper controls including untreated, vehicle only, positive controls, and non-functional analogs will be included in each experiment. When possible, viral stocks will be generated from cDNA clones and sequence confirmed. Mouse experiments will be used to assess *in vivo* efficacy of lead molecules against a number of alphaviruses. In the Vertebrate Animals section, we provide sample size estimates based upon power calculations. In general, animal experiments will be performed in small batches to ensure reproducibility. WT mice will be purchased from commercial vendors to ensure quality and reproducibility. Mice will be randomized into experimental groups to reduce bias and the analysis will be blinded. Equal numbers of male and female mice will be used to monitor for sex-based differences. Furthermore, key findings can be confirmed by parallel or orthogonal experiments in multiple laboratories. Reporting of experimental data will be transparent to fully allow reproducibility by others. Results will be analyzed by parametric and non-parametric analyses, depending upon data distribution, making appropriate corrections for multiple comparisons.

D. COOPERATIVE PLAN AND RELATIONSHIP TO CETR (AD3C). Our multidisciplinary team includes medicinal chemists, structural biologists, computational biologists, and virologists that provides cutting edge technologies to develop lead molecules. Interactions with other projects will facilitate the testing of each project's lead compounds against related and unrelated viruses. Together with Southern Research (SR) and the Emory Institute for Drug Development (EIDD), which are leaders in drug discovery and Med Chem, our AD3C project is also partnered with Gilead Sciences to help with translational application and product development.

E. BACKGROUND AND PRELIMINARY DATA. Prior to the start of the AD3C CETR, SR performed an HTS campaign of 384,000 compounds against VEEV, which identified an initial set of leads for the Medicinal Chemistry and Lead Development Core (MCLDC). These compounds were analyzed for structural liabilities using the Pan Assay Interference Compounds (PAINS) filter to identify problematic chemotypes[9, 10]. The remaining hits were analyzed for structural similarities using clustering analysis that represented chemical diversity and novel structures. Hits were ranked according to antiviral activity, cytotoxicity, selectivity index ($SI=CC_{50}/EC_{50}$), and structural desirability. A set of 35 compounds were selected and reconfirmed in antiviral and cell cytotoxicity assays against both VEEV and CHIKV using primary human dermal fibroblasts (NHDF). Based on these studies, **SRI-33394** for VEEV and **SRI-33366** for CHIKV were selected for further development. In addition, a new CPE-based HTS screen of 197,000 unique compounds against CHIKV and VEEV was completed. Using an activity threshold, samples were identified as active and retested in a CPE assay and for direct cytotoxicity effects. Using a CHIKV NanoLuc Luciferase Reporter (CLNLR)-based assay and a VEEV CPE-based assay, several hits with EC_{50}<10µM and SI (CC_{50}/EC_{50}) >10 for each virus were confirmed. After PAINS filtering and cluster analysis, **11** compounds for CHIKV and **15** compounds for VEEV were selected (EC_{50}<10µM and CC_{50}>30µM) for confirmatory assays using viral titer reduction (VTR) and dose response assays.

1. Assay development and utilization.

(a) CHIKV–Virus Linked Nanoluc Assay (CLNLA). AD3C engineered a human telomerized fibroblast line, THF-Neo-ΔIRF3, which lacks interferon regulatory factor 3 and is incapable of producing type I IFN. This prevents identification of compounds that inhibit viral replication through IFN-dependent activity. In this assay, cells were added to 384-well assay plates pre-drugged with test compounds. Cells were infected with CHIKV 181-25 encoding a NanoLuc reporter (CLNLR). Uninfected cells served as the low signal control (100% inhibition virus replication) and virus-infected cells served as the high signal control (0% inhibition). At 48 hpi, culture media was evaluated by VTR assay (described below) and cell lysates were assessed for luminescence using NanoGlo (Promega). This assay was used to evaluate structure-activity relationships (SAR) by Assay Core.

(b) VEEV–CPE Assay. THF-Neo-ΔIRF3 cells were mixed with the BSL2 attenuated strain VEEV TC-83 and plated in 384-well assay plates pre-drugged with test compounds. DMSO was maintained at 0.5% for all wells. Uninfected cells served as the high signal control (100% inhibition of CPE) and virus-infected cells served as the low signal control (0% inhibition). At 72 hpi, culture supernatants were evaluated by VTR assay. Cell viability was determined using CellTiter Glo (Promega). This assay was used to evaluate SAR by Assay Core.

(c) Cell Viability Assay. THF-Neo-ΔIRF3 cells were added to 384-well plates pre-loaded with compounds. Cells only (100% viability) and cells treated with a cationic detergent (0% viability) served as negative and positive controls, respectively. At 72 h post-plating, viability (CC_{50}) was assessed using Cell Titer-Glo.

(d) Virus Titer Reduction (VTR) assay. VTR used supernatants from the CLNLA and VEEV-CPE assays (10 and 1 µM doses). Virus-containing supernatant was diluted and dispensed to 5 replicate wells of THF-Neo-ΔIRF3 cells to produce an 8-log range of test wells. At 48 hpi, expression of NanoLuc (NanoGlo) or detection of CPE (CellTiter Glo) was used to detect the presence of virus. Wells scored positive if the NanoLuc/CPE signal was 3 times different than the median of the control. $TCID_{50}$ was calculated as the concentration at which the number of virus positive wells was equal to 2.5 (50% of the 5 wells plated at each concentration). Number of infectious virus particles (IVP) was calculated as $TCID_{50}*100^{11}$. This was based on 2.5 IVP/25 µl or 100 IVP/ml). VTR was calculated as $\log_{10}$ (Virus controls IVP/Compound IVP).

2. Lead generation and optimization.

(a) CHIKV: 11 HTS hits for CHIKV were selected for hit to lead generation and optimization studies, and two compounds (**SRI-33001 and SRI-36767**) were prioritized based on high VTR (>5 logs), EC_{90} <1.0µM, CC_{50}>30µM and structure desirability (**Table 1**). These compounds and a previous CHIKV benzoannulene lead (**SRI-33366**) were analyzed in ADME assays to determine solubility at pH 7.4, mouse and human liver microsomal (MLM & HLM) stability, and LogD. These data, combined with EC_{90}, CC_{50}, and VTR results, guided medicinal chemistry optimization. Target values for lead compounds

(b) (4)

(b) (4)

[redacted]

(b) (3) (A), (b) (6) [redacted]

(b) (3) (A), (b) (6) [redacted]

(b) (4) [redacted]

(b) (4) [redacted]

(c) Novel Nucleoside Inhibitors of Alphavirus Replication: Nucleoside analogs (Nuc) play an important role in the treatment of viral infections by acting as competitive, alternative substrates for virally-encoded polymerases. Importantly, Nuc toxicities are well understood, generally being derived from off-target inhibition of host polymerases, which can be identified early in drug development. EIDD has rationally designed a library of Nucs that target viral RNA-dependent RNA polymerases (vRdRps). Our lead compounds **EIDD-1931**[8] and **EIDD-2749** are potent inhibitors of alphavirus replication (**Fig 5** and **Table 3**) and **EIDD-1931** shows protective efficacy against CHIKV induced tenosynovitis *in vivo* (**Fig 12**). We will explore the anti-alphavirus activity of **EIDD-1931** and **EIDD-2749** and evaluate the EIDD Nuc collection to identify additional leads.

F. SCIENTIFIC APPROACH

SPECIFIC AIM 1. To chemically optimize inhibitors of alphavirus replication.

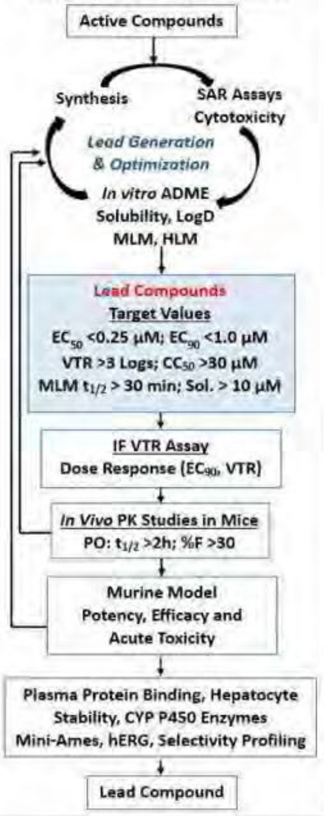
Rationale. One goal is to develop compounds with pan-alphavirus inhibitory activity for clinical application. Through HTS campaigns, we identified small molecule leads against CHIKV and VEEV that show broad antiviral activity against multiple alphaviruses (**Table 3**). Our preliminary data also indicate that the nucleoside analogs **EIDD-1931** and **EIDD-2749** are potent broad inhibitors of alphaviruses *in vitro* and CHIKV *in vivo* (**Table 3**, **Fig 12**). Since nucleoside analogs are well-established antiviral agents in clinical use, we will evaluate the EIDD Nuc library for additional compounds that block alphavirus replication. Hits will be prioritized based on activity/toxicity profiles and up to 5 hits will be pursued further for lead optimization in our product development pipeline. Mode of action, antiviral breadth, and cell type specificity for lead compounds will be performed in Aim 2. The optimized lead compounds with acceptable pK and bioavailability profiles will be tested in Aim 3 for antiviral efficacy in mouse models of alphavirus infection and disease.

Drug Development Pipeline

(1) EIDD Nucleoside Collection. The EIDD Nuc collection consists of 1,000+ compounds rationally designed as competitive, alternative substrate inhibitors of vRdRps. The collection of compounds contains ~800 ribonucleosides and 5'-phosphate prodrugs as well as 200 5'-triphosphates. The collection of compounds represents Nuc analogs that feature modified cyclic and acyclic carbohydrate scaffolds coupled to natural nucleobases, modified nucleobases able to participate in canonical Watson-Crick or Hoogsteen base pairing, and non-canonical nucleobases. Most of the compounds have been screened against lymphoid and hepatocyte cells and found to be non-cytotoxic. We will utilize our CHIKV-VLNLA and VEEV-CPE assays (described above) to evaluate the EIDD Nuc compounds for antiviral activity. The assays will be performed in THF-Neo-ΔIRF3 cells, which contain sufficient levels of kinases for triphosphate conversion. Active hits will be further analyzed for *in vitro* toxicity and antiviral activity against WT strains of CHIKV, VEEV, and other alphaviruses (MAYV, RRV, ONNV, EEEV, Una). EC_{50} will be determined as described above using 3-fold serial dilutions of hit compounds. Similarly, CC_{50} values in uninfected cells will be determined over the same dilution series by measuring ATP. A SI will be determined to prioritize hits (SI$\geq$5) for further development. Chemical assessments of drug-like properties will be used to identify up to 5 of the most active leads that will feed into our compound chemical optimization pipeline.

(2) SAR, Lead Development, and Optimization. We identified 3 compounds amenable to chemical optimization for CHIKV and 2 compounds for VEEV as well as **EIDD-1931** and **EIDD-2749**. The compound progression pathway for lead optimization is illustrated in **Fig 6**. An iterative design-synthesis-testing process will be used to identify potent analogs with drug-like (i.e., physicochemical and pharmacokinetic) properties and to develop SAR within a chemical series. Analogs of hit compounds will be designed to improve potency and efficacy in the SAR-driving assays (CHIKV-VLNLR/VTR and VEEV-CPE/VTR) using THF-Neo-ΔIRF3 cells. In addition, as part of this Aim, compounds will be tested for activity against WT strains of CHIKV and VEEV, while the breadth of antiviral activity will be evaluated in Aim 2. Compounds with EC$_{50}$<0.25 μM will be evaluated in microsomal stability, solubility, and LogD studies. Some inactive compounds also will be evaluated for ADME to guide design and to understand effects of chemical modifications on solubility or microsomal stability. This iterative process will be continued until target values are achieved (**Fig 6**). The compounds with potent antiviral activity and an acceptable ADME profile will advance. We will strive to achieve balanced antiviral activity and drug-like properties. Particularly for VEEV, a critical objective is to identify compounds with acceptable endothelial permeability because active compounds may need to cross the blood-brain-barrier (BBB) for physiological effect. Additional parameters will be evaluated during the optimization process, such as calculated polar surface area (tPSA=40–70) and experimental logD=2–4. *In silico* model predictions of brain permeation will be utilized during analog design[15]. *In vitro* permeability assays to determine absorption, permeation and transport of compounds into tissues and across cell membranes (PAMPA, MDR1-MDCK or Caco2) will be performed on active compounds. P-glycoprotein (PgP) efflux is the most significant limitation to BBB penetration and important to assess early for a series using an *in vitro* PgP assay (P$_{app}$≥10e^{-6}cm/s, Efflux ratio ER≤2.5)[16].

Figure 6. Lead Optimization


Active Compounds
Synthesis
SAR Assays
Cytotoxicity
Lead Generation
& Optimization
In vitro ADME
Solubility, LogD
MLM, HLM
Lead Compounds
Target Values
EC$_{50}$ <0.25 μM; EC$_{90}$ <1.0 μM
VTR >3 Logs; CC$_{50}$ >30 μM
MLM t$_{1/2}$ > 30 min; Sol. > 10 μM
IF VTR Assay
Dose Response (EC$_{90}$, VTR)
In Vivo PK Studies in Mice
PO: t$_{1/2}$ >2h; %F >30
Murine Model
Potency, Efficacy and
Acute Toxicity
Plasma Protein Binding, Hepatocyte
Stability, CYP P450 Enzymes
Mini-Ames, hERG, Selectivity Profiling
Lead Compound

Compounds will be prioritized and selected for *in vivo* PK studies in mice based on activity, potency, and *in vitro* ADME [EC$_{90}$<1 μM; CC$_{50}$>30 μM; VTR>3 logs at 10 μM; MLMt½>60 min; Sol>10 μM]. PK studies will preferably be done orally (PO) and if acceptable exposure of a compound is not obtained via PO administration (oral bioavailability F>30%, half-life>2h), other routes of administration [intravenous (IV), subcutaneous (SC) or intraperitoneal (IP)] can be investigated. If the PK data warrant further studies, iterative design and synthesis will continue. Compounds that have acceptable *in vivo* PK data will advance into animal studies as part of Aim 3. We will target compounds for VEEV with a permeability coefficient >10e^{-6}cm/s, bioavailability>20% and a brain to plasma (B/P) ratio ≥0.4. All of these properties will serve as guidelines to determine *in vivo* candidates. Compounds that are efficacious in animals should also show at least a 5-fold greater compound concentration in plasma than the measured EC$_{90}$.

(a) CHIKV inhibitors. Design of new CHIKV inhibitor analogs will be performed on **SRI-36498** [EC$_{90}$ =0.98μM; VTR=4.9 logs at 10μM; MLMt½=57min; Sol=0.5μM], **SRI-40469** [EC$_{90}$=0.25μM; VTR=4.7 logs at 10μM; MLMt½=56.9min; Sol=1μM] and **SRI-40507** [EC$_{90}$=0.50μM; VTR=7.4 logs at 10μM, MLMt½=5.3 min; Sol=65μM] to improve activity and drug-like properties. Compounds will be tested for their activity, potency, and cytotoxicity to generate SAR data. The design of new compounds will follow the Lipinski's 'rule of five' to evaluate 'drug-likeness' as well as based on calculated drug-like properties such as solubility (LogS), lipophilicity (clogP) and StarDrop™-CSL for metabolically unstable areas. These criteria will be used as a guideline for designing and prioritizing targets. The predicted metabolic sites of liability by StarDrop™ CSL calculations for **SRI-40507**, **SRI-40469** and **SRI-41171** (analog of 40469 with better CSL value) are 0.5205, 0.8835 and 0.7603, respectively. Solubility, LogD, and microsomal stability will be determined experimentally on compounds to guide the design of newer analogs with more favorable PK properties. Some areas for chemical modifications are shown in **Fig 7** in which a set of compounds at each indicated site will be designed and synthesized. A goal for the **SRI-36498** series is to improve solubility (>10 μM). Based on SAR generated and calculated LogS values, specific analogs in the indicated areas of **SRI-36498** (**Fig 7A**) will be tested for antiviral activity. As discussed in the preliminary studies section, **SRI-36498** is soluble up to a 20 mg/kg dose in 2%DMSO/3% solutol in 30%HP-β-CD. The aim is to increase solubility to at least 80 mg/kg for mouse studies to reach 5X the EC$_{90}$. For **SRI-40469**, we have achieved potency (EC$_{90}$=0.25 μM) and microsomal stability (MLMt½=56.9 min), but there is a need to increase solubility. Iterative design, synthesis, and testing will be carried out to generate SAR and increase solubility by introducing polar groups in the molecule (**Fig 7B**). The major shortcoming of **SRI-40507** is the thioacetamide group, which leads to poor

(b) (4)

metabolic stability and possible liver toxicity[17, 18]. We will start medicinal chemistry by cyclizing the thioacetamide group to pyrimidin-2-one ring, resulting in a thienopyrimidin-2-one (**Fig 7C**). Further modification of the thienopyrimidin-2-one scaffold will be done to attain target values of potency, efficacy, and PK.

(b) VEEV inhibitors. Some areas of chemical modifications for **SRI-36129** [EC_{90}=1.3μM; VTR=3.0 logs at 10μM; MLMt½=21.6min; Sol=51.5μM] and **SRI-36426** [EC_{90}=0.92μM; VTR=6.0 logs at 10μM; MLMt½=5.4min; Sol=31.8μM] are shown in **Fig 8**. Brain exposure for VEEV compounds can be enhanced by reducing H-bonds, molecular weight, PgP efflux, metabolism, and plasma protein binding, and/or by increasing Log P. Compounds that meet the desired activity criteria will be evaluated for solubility, microsomal stability and LogD.

(b) (4)

(c) Nucleoside Analogs. The optimization process for Nuc-scaffolds will follow a similar pathway as shown in **Fig 6**. The process begins with an iterative cycle of analog design, synthesis and biological testing. CLNLA and VEEV-CPE assays will be performed with all Nuc compounds. Those showing suitable activity (EC_{50}<5 μM) and a selectivity SI>10 will advance to pharmacological assessments. Focused libraries will be prepared utilizing late-stage glycosylation between modified nucleobases and modified ribofuranoside intermediates. Nucs that are inactive in cellular infection assays will be further assessed in cellular anabolism assays to identify the anabolic blocks potentially responsible for lack of cellular efficacy. The EIDD has established sensitive and selective LC/MS/MS methods for the quantitative determination of intracellular levels of parent Nuc and prodrug conjugates as well as the 5'-monophosphate, 5'-diphosphate, and 5'-triphosphate metabolites in samples from *in vitro* and *in vivo* studies. Prodrug conjugates of inactive Nucs will be prepared to overcome the problematic step of achieving 5'-triphosphate concentrations necessary for activity in cellular assays. PK and distribution studies will be conducted on Nucs that have met the established activity, selectivity, and stability selection criteria, in the animal species used in the *in vivo* efficacy model. PK studies have been completed for **EIDD-1931** and **EIDD-2801** in mice, rats, dogs, and non-human primates and demonstrate good bioavailability.

(b) (4)

(d) Synthesis and Data Management. The synthesis of compounds follows literature precedents and methods developed by SR chemists. As an example, the synthesis of **SRI-40469** is illustrated in **Fig 9**. We anticipate that ~200 analogs will be synthesized each of Years 1-3. During Years 4-5, it is expected that compounds with improved ADME profiles will have been identified and less chemistry effort required. Approximately 25 mg of compounds will be prepared (>95% purity) for SAR and the structure and purity will be confirmed by NMR, Mass Spectrometry, and HPLC. Gram quantities of selected compounds for animal experiments will be synthesized (**Core C**) when necessary. Dotmatics™, an informatics and compound registration database at SR, will be used for ADME data capture, analysis, and storage of biological activity on all analogs, with each new compound given a unique identifier (i.e., SRI number).

(3) *In vivo* ADME. The MDCC will coordinate all *in vivo* PK studies of compounds using well-established protocols *at Pharmaron, Inc*. Six-to-eight compounds will be evaluated based on acceptable *in vitro* ADME properties, antiviral potency, and efficacy. The PK profile will be assessed in C57BL/6 mice following single PO administration (e.g., dose: 5 mg/kg, vehicle: 2% DMSO/3% solutol in water). Properties, such as plasma half-life ($t_{1/2}$), peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), systemically available fraction of a compound (bioavailability %F), clearance (CL), volume of distribute (V_d), and area under the curve (AUC_{inf} and AUC_{last}) will be determined. For VEEV compounds, *in vivo* B/P ratio to extrapolate unbound brain drug concentration also will be determined. Compound dose and frequency of administration for *in vivo* efficacy studies in murine models will be calculated based on PK properties. If acceptable exposure of compound is not achieved by the above formulation, other formulations or administration routes will be used. Each study will include 8 groups of 6 WT C57BL/6 mice, with each group euthanized at either 15, 30 min, 1, 2, 4, 8, and 24 h. Each mouse within the groups will be given a single dose of test compound. Control mice will be given vehicle only. Individual animals will be observed and weighed, and plasma collected for analysis by HPLC-MS/MS to determine the concentration of intact compounds and metabolites, as appropriate. Relevant PK parameters and oral bioavailability will be calculated for each test compound via WinNonlin®.

(4) Off-Target Interactions. Compounds with acceptable PK profiles (%F>30%, $t_{1/2}$>2h) and *in vivo* efficacy will be analyzed for off-target interactions. For example, assessment of the capacity of a compound to inhibit CYP450s enzymes is important as co-administration of compounds may result in one or both inhibiting the other's metabolism. This may affect plasma levels *in vivo* and potentially lead to adverse drug reactions or toxicity. Specifically, the six major drug metabolizing CYP450s in humans will be tested: CYP-1A2, -3A, -2D6, -2C9, -

2C19, and -2E1. The compounds will be tested in high-throughput panels for off-target effects (e.g., Panlabs, 240 host-derived targets) to evaluate a wide range of targets, including GPCRs, kinases, and nuclear receptors. The desired profile for progression will have <50% inhibition on all targets at 10 μM. Compounds will be tested in mini-Ames mutagenicity tests [mutagenic potential of new drugs using two strains of *Salmonella typhimurium* (TA98 and TA100)] and evaluated for hERG potassium channel activity (cardiac risk) (Low: IC_{50}>10μM; Moderate: 1μM<IC_{50}<10μM; High: IC_{50}<1μM). Metabolite identification studies can be performed to address designing around any identified liabilities.

Expected Outcomes and Alternate Strategies. We will continuously evaluate progress and timelines and make **Go/No-Go** decisions as we execute the described medicinal chemistry strategies. Our strategy should result in lead candidates with acceptable drug-like properties to initiate animal studies in late Y1 or Y2. Thus, for a compound to be nominated for animal studies, it will have met designated *in vitro* criteria such as activity, potency, solubility and microsomal stability, as well as *in vivo* criteria such as bioavailability and half-life. It is common that optimization for one parameter negatively impacts another parameter and various modifications will be evaluated to achieve a balance between the desired potency and preferred drug-like properties. We are cognizant of this and will optimize efficiently and effectively, using our iterative medicinal chemistry and PK knowledge to put forth the best inhibitor(s) for further development. It is possible that compounds or chemical series cannot be optimized for acceptable drug-like properties with the preferred biological activity. We will make **Go/No-Go** decisions as we execute lead optimization and will discuss issues or concerns with the Research Project Site Leader/Team, the Administrative Core, and Scientific Advisory Committee.

SPECIFIC AIM 2. To determine the antiviral mechanisms of inhibitors of alphavirus replication.

Rationale. Lead compounds will require more thorough validation and characterization to assess their eligibility for advancement to downstream SAR and *in vivo* models (Aim 3). This will involve examination of the following criteria for individual lead compounds: 1) Breadth of virus species against which antiviral effects are evident; 2) Efficacy and toxicity in multiple cell types; 3) Molecular basis of virus inhibition; and 4) Ease with which resistance mutants arise and characterization of their properties.

Experimental Approach.

(1) Cell Type Specificity and Cytotoxicity. Alphaviruses replicate in numerous vertebrate tissues and cell types *in vivo*[19] as well as in mosquitoes. We will evaluate the importance of cell type for compound inhibitory activity using mosquito, human and murine cell lines representative of tissue types for which the viruses exhibit tropism (C6/36 mosquito cells, NHDF, synovial fibroblasts (SF)[20], neuronal-derived cell lines SK-N-MC[21], myoblasts and myofibers[22-24], and primary myeloid-derived dendritic cells and macrophages[25]). Cells will be treated with increasing concentrations of inhibitor (as above) and infected using an MOI of 1-10 PFU/cell. Culture supernatants collected at 12, 24, and 48 hpi will be analyzed by plaque assays to quantify yields of infectious virus and determine EC_{90} values for each cell type. Cytotoxicity (CC_{50}) will be determined by metabolic assays as described above to calculate the SI for each compound in discrete cell types.

(2) Spectrum of Antiviral Activity. We will determine the phylogenetic breadth of alphavirus species against which efficacious compounds are inhibitory. First, we will verify that compounds are effective against naturally occurring, geographically diverse strains of CHIKV and VEEV representative of the major genotypes and subtypes. These viruses exist already in our laboratories or will be obtained from the Arbovirus Reference Collection at the UTMB-Galveston. EC_{90} dose response assays will be performed in THF-Neo-ΔIRF3 cells with MAYV, ONNV, RRV, Sindbis virus (SINV), Semliki Forest virus (SFV), Una virus, and South American strains of EEEV that are not select agents. Our group has extensive experience working with numerous alphaviruses and the (b) (3) (A), (b) (6) has Select Agent approval for working with the virulent IAB VEEV subtype strain VEEV-TRD. We have observed that most of our lead compounds are effective against CHIKV and VEEV; and we predict that these compounds also will inhibit the replication of other alphaviruses, though it is possible that we will identify inhibitors that are active against subsets. Active compounds also will be tested in Projects 1, 2, and 4 for activity against other virus families. *Priority will be placed on compounds with activity against >2 alphaviruses or compounds that inhibit coronaviruses, influenza virus, and/or flaviviruses*.

(3) Mechanism of Action Studies.

(a) Time of Addition: Fluorescence microscopy-based time of addition studies will be used to identify the kinetics of inhibitory activity. CHIKV-$_{181\text{-}25}$mKate or CHIKV-$_{AF15561}$mKate (BSL2 and BSL3 strains, respectively), recombinant viruses that express mKate, will be used to monitor viral infection. We will infect six replicate wells in 96-well black wall plates seeded with THF-Neo-ΔIRF3 cells. Cells will be treated with compounds at -2, 0, 2, 4, 6, 8, and 12 hpi. At 24 hpi, cells are fixed and stained with DAPI. Fluorescence images (DAPI, mKate) will be captured using a CellInsight CX5 High Content Screening Platform (OHSU). Based on the level of fluorescence intensity, the system software counts the number of DAPI positive objects (cellular nuclei) in each well, and mKate expression is quantified in three different ways that include the number of mKate positive cells (mKate spot count), the total mKate positive area/mKate positive spot, and total mKate positive spot intensity/well. The number of mKate positive cells and fluorescence intensity is indicative of the level of CHIKV-mKate infection. We predict that compounds that are effective at: -2 or 0 hpi block virus attachment/entry; 2-4 hpi block nsP synthesis, viral RNA replication or translation; and 6-10 hpi block viral assembly or release. These predictions will be assessed further using the assays described below.

(b) Binding/Entry: Compounds that require cell pre-treatment to inhibit replication may act at the stage of virion binding and/or entry. Since alphavirus genomic RNA is infectious when introduced into a permissive cell by transfection or electroporation, we can use this method to bypass binding and entry to determine whether a candidate molecule is still inhibitory[26]. To ensure that we are only measuring the impact of the drug on the initially transfected cells rather than downstream effects on virus production, we will use single cycle CHIKV or VEEV replicon RNAs in which the viral structural proteins have been replaced by nLuc. Cells will be treated with the candidate compound or vehicle control and transfected with CHIKV- or VEEV-nLuc replicon RNA. We will then measure nLuc activity at 12 h under each condition. If a compound reduces nLuc expression, this would indicate that the compound inhibits viral replication at a post-binding and entry step of replication. However, if the compound does not affect nLuc expression following electroporation, binding assays[27] and viral fusion assays[28] would be performed to measure the impact of the compound on viral binding to cells or virus entry.

(c) Viral RNA/Protein Synthesis: Based on preliminary data with our current lead compounds, we predict that the majority of efficacious compounds block viral RNA synthesis or protein translation. To evaluate these possibilities, we will assess the effect of compounds on viral genome replication using qRT-PCR to measure synthesis of positive- and negative-strand RNA. For quantification of negative-strand RNA, we will use a sequenced-tagged negative-strand specific primer for the RT step and appropriate strand-specific controls[29]. To measure production of the alphavirus subgenomic mRNA, we will analyze the ratio of genomic to subgenomic RNA synthesis in the presence of compounds by qRT-PCR[30] and Northern blotting using probes directed against the viral structural genes. Translation and processing of the viral nonstructural proteins (NSP) is required for viral RNA synthesis. As such it will be necessary to determine whether a decrease in viral RNA is due to an NSP- rather than inhibitor-dependent function. We will thus examine levels of NSP expression by western blotting using existing antibodies against nsP3 of CHIKV[26] or nsP2 of VEEV[31]. Since NSP translation is required for *de novo* viral RNA synthesis, a general decrease in viral RNA and NSP levels would distinguish whether the inhibitor was acting at the level of viral RNA synthesis or by interfering with viral NSP translation. If we observe a direct effect on the translation of the viral NSPs, we would evaluate the impact of the candidate compound on the initiation and/or rate of viral protein translation using polysome gradients[31] and pulse chase studies.

(d) Assembly and Release: If antiviral effects on virus entry or macromolecular synthesis are not detected in the presence of a target compound, we will evaluate the effect of compounds on the expression and transport of the alphavirus structural proteins, as well as virus assembly and release. Overall levels of CHIKV or VEEV capsid, E2, and E1 protein levels and processing will evaluated by quantitative immunoblotting (IB) in the presence and absence of target compound. The kinetics of structural protein synthesis will be measured by immunoprecipitation following pulse chase radiolabeling. We also can determine whether viral glycoproteins are transported to the cell surface by IFA staining and flow cytometric analysis for E1 and E2 glycoprotein expression. If surface expression is decreased, we will evaluate both the stability and maturation of the E1 and E2 glycoproteins by pulse radiolabeling the proteins, and by testing the rate at which the viral N-linked glycans on the labeled proteins become resistant to endoglycosidase-H, to evaluate protein maturation and transport from the ER/Golgi. If viral structural protein production and cellular expression is normal, we will measure the overall levels and rate of viral release in the presence or absence of the candidate compound. Lastly, if viral release is normal, but yields of infectious virus are reduced, we will perform specific infectivity assays to determine whether the *de novo* synthesized virus produced in the presence of the compound is less infectious (high particle:PFU ratio).

(b) (4)

[redacted]

(e) Structure-based Design.

(i) nsP3 macrodomain. Resistance against **SRI-34963** mapped to residues Gly117 and Asp119 of CHIKV-nsP3 macrodomain. We have determined the structure of the nsP3 macrodomain at 1.46 Å by X-ray crystallography, which allows us to study inhibitor interactions in the nsP3 structure.

To determine the feasibility of finding inhibitors in nsP3 crystals soaked with inhibitors, ADP-ribose (ADPR), a natural ligand of nsP3 macrodomain[32], was used to optimize the soaking condition. ADPR was clearly identified at the ADPR binding site in the nsP3 macrodomain (**Fig 10a**). DMSO does not affect the binding of ADPR to the nsP3 macrodomain. Recently, *in silico* docking identified a fragment compound, isobutyl pyrimidone, as binding within the ADPR pocket. **Fig 10b** shows the empirical nsP3 macrodomain crystal structure and tight binding of isobutyl pyrimidone, which is a moiety within our lead **SRI-40507**. Structural modeling indicates **SRI-40507** shares similar binding mode (**Fig 10c**). Once potent inhibitors are developed by SAR studies of **SRI-40507** and **SRI-36498**, these compounds will be soaked into nsP3 macrodomain crystals. Alternatively, inhibitors can also be co-crystallized with the nsP3 macrodomain. The structural information of the nsP3-inhibitor complex will be used to design more potent and refined analogs.

(ii) nsP2 helicase domain. The alphavirus nsP2 protein contains N-terminal RNA helicase activity and C-terminal cysteine protease activity. In preliminary drug resistance and reverse genetic studies, we found that VEEV with an amino acid substitution at nsP2 Y101 (Y101S) is resistant to **SRI-36129**. Remarkably, this mutation was previously reported to confer VEEV resistance to quinazolinones[33], suggesting that this region of nsP2 is a robust therapeutic target for drug development. Although the structure of the C-terminal protease domain of VEEV nsP2 was determined[34], there is no structural information available for the N-terminal helicase domain. To facilitate the current drug discovery effort for VEEV, we will determine the crystal structures of VEEV nsP2 and the nsP2 helicase domain in the presence or absence of identified inhibitors.

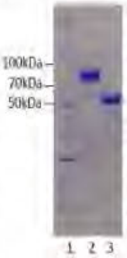


Fig 11. SDS-PAGE of purified full-length VEEV nsP2 (lane 2) and nsP2 helicase domain (residues 1-463; lane 3).

The full-length nsP2 protein and the helicase domain of nsP2 (residues 1-464) have been expressed in *E. coli* and purified to homogeneity by nickel-NTA affinity and size-exclusion chromatography (**Fig 11**). The goal of our structural biology efforts is to provide proteins for helicase activity assays[35] and to help develop SAR of selected compounds by structure-based compound design. Purified nsP2 protein will be used in the helicase activity assay to identify specific inhibitors from the SAR studies. We will determine the structure of the helicase domain, which will be used to design and develop lead compounds. Selected inhibitors identified from the nsP2 screen will be co-crystallized with the helicase domain or the full-length nsP2. Alternatively, if the crystal structures of nsP2 proteins are determined, soaking the inhibitors into the nsP2 crystals will be considered. The structures of the nsP2-inhibtor complexes should provide structural information of inhibitor interactions in binding sites, which will aid design of more effective inhibitors.

In addition, biochemical and/or cellular thermal shift assays will be used to quantify changes in protein thermal denaturation temperatures associated with antiviral compound binding[36]. These assays are useful for quantifying differences in the binding between compound analogs and for validating target identification in cells. Thermal shift data will be correlated with SAR results and structural data obtained through crystallography.

(f) Computational Chemistry Strategy. Computer-aided drug design will be applied to the discovery and development of inhibitors of CHIKV and VEEV. For example, structure-based compound design will be performed utilizing our crystal structure of the CHIKV nsP3 macrodomain. One orthosteric binding site (ADP-ribose binding site) and one allosteric binding site have been defined (**Fig 10**) based on the crystal structure of the macrodomain/ADP-ribose complex and the resistance mutations (nsP3 Asp119 and Gly117) identified by our group, using SiteMap of Schrödinger Small Molecule Discovery Suite 2015. Induced-fit docking implemented in Schrödinger will be used to estimate the thermodynamic binding affinity to the nsP3 macrodomain when designing new compounds. Moreover, potential allosteric effects will be examined via classical molecular dynamic (MD) simulations using the GPU-accelerated version of AMBER Dynamic Package 2016. The nsP3 macrodomain in *apo* state and in complex with a selected number of compounds will be tested in MD simulations. The protein systems will be solvated using TIP3P explicit water model and treated with AMBER Force Field 2012 as well as periodic boundary condition. Similar computational approaches will be applied to the structures of nsP2 and the nsP2 helicase domain, which will be further informed by the **SRI-36129** resistance mutation identified at nsP2 Y101. Experiments combined with these computational approaches should provide an in-depth understanding of the binding modes of active compounds.

(g) Resistance. We have used the generation and analysis of viral mutants resistant to two of our antiviral leads to identify the molecular basis of inhibitory mechanisms (i.e., nsP2 helicase for **SRI-36129** and nsP3 macrodomain for **SRI-36498**). We will use this approach to determine resistance phenotypes and ease of resistance development by culturing CHIKV or VEEV (and additional drug-sensitive alphaviruses) infected fibroblasts in the presence of compounds at the EC_{90} for multiple passes (5-10). Mutant virus genomes will be deep sequenced to identify specific mutations that correlate with the generation of resistance. These mutations will be inserted into CHIKV or VEEV cDNA clones to define mutations that confer drug resistance. Existing cDNA clones (RRV, MAYV, ONNV) or newly generated cDNA clones for other alphaviruses will be used to assess the capacity of specific mutations to confer drug resistance across alphavirus species. Viral mutant fitness will be tested by assessing viral growth in human and insect cells and in mice (SA3). The identification of specific mutations that confer resistance will help elucidate antiviral mechanisms of action. In addition to targeting the vRdRps or by acting as chain terminators, nucleoside analogs may cause lethal mutagenesis[8]. To test this, we will deep sequence vRNA released from cells treated with nucleoside analogs and quantify the mutation frequency and types of mutations across the viral genome, this analysis could also be used for *in vivo* samples.

(4) Synergistic vs. Additive Effects. We will determine whether combinations of two or three alphavirus antivirals have synergistic or additive inhibitory effects. We will utilize the methods developed by Prichard et al. (**Project 4**) that evaluate synergistic effects of combinations of antivirals through computational modeling of empirical data (MacSynergy-II)[37]. For this study, we will adapt our CHIKV-VLNLA and VEEV-CPE assays to include matrices of drug combinations in 96-well assay plates. Briefly, theoretical additive interactions are calculated from dose-response curves for individual drugs, and then the additive interactions are subtracted from the experimental results from drug combinations to reveal greater or less than additive effects that would be indicative of drug synergy or antagonism. These procedures would also be applied to cytotoxicity assays to determine whether certain drug co-treatments increase toxicity. Synergistic or additive compound combinations will be assessed for enhanced *in vivo* efficacy (SA3.B) and for reducing resistance emergence (SA2.3g).

Expected Outcomes and Alternate Strategies. Defining the mechanism of action for antiviral compounds will facilitate development of improved compounds with higher specific activities against the viral target. Furthermore, if multiple compounds act at different replication stages, we may be able to use this information to develop combination therapy that will: 1) Exhibit improved *in vivo* efficacy; and 2) Impede emergence of resistance mutants. We do not anticipate any serious technical problems with the proposed approaches since the described methods are well established in the laboratories of the investigators. However, it is possible that some compounds will not yield escape mutants. Nevertheless, our detail investigations should identify the step of the viral replication cycle inhibited by the compound.

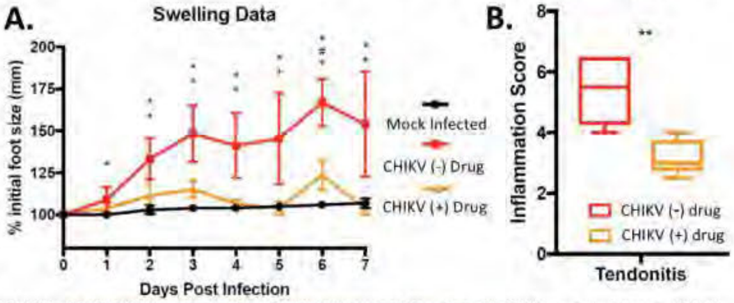


Fig 12. EIDD-1931 Protects from CHIKV-Induced Disease. C57BL/6J mice (n=5) received EIDD-1931 (35mg/kg) or diluent i.p. at 12hrs prior to CHIKV inoculation (10^3 PFU in left rear footpad; n=5/group). (**A**) Left footpad swelling was assessed by calipers daily. ⁺<0.05 by Tukey's Multiple Comparisons Test: *CHIKV (-) vs. Mock; ⁺ CHIKV (+) vs. CHIKV (-); # CHIKV (+) vs Mock. (**B**) Mice were harvested at 7 dpi and tendonitis assessed using our established pathology scoring system (0 = no inflammation, 1-2=very mild, 3-4=mild, 5-6=moderate, 7-8=severe, and 9-10=very severe inflammation). ** p <0.01 by two tailed, unpaired t-test.

SPECIFIC AIM 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease *in vivo*.

Rationale. Our lead compounds show promising antiviral activity *in vitro*, with some (**EIDD-1931**) already progressing to *in vivo* testing (**Fig 12**). Therefore, following SAR, compounds with appropriate activity and PK properties will be evaluated using our established murine models that recapitulate alphavirus-induced encephalitis (VEEV) and arthritis (CHIKV) observed in humans. As CHIKV causes severe acute and chronic arthritis in humans, CHIKV studies will use an immunocompetent C57BL/6 mouse model of CHIKV-induced inflammatory arthritis and joint swelling[26, 38, 39] that progress to chronic disease characterized by persistence of viral RNA in joint tissues as well as chronic synovitis and tenosynovitis. Antivirals that show good prophylactic and therapeutic efficacy in this model also will be tested in type I interferon receptor deficient mice (*Ifnar1*$^{-/-}$), which are highly susceptible to CHIKV disease. In addition, we also can test compounds using our established murine models of RRV and MAYV arthritic disease. VEEV is highly virulent in WT mice via both peripheral and intranasal routes[40], and therefore, we will test lead inhibitors for *in vivo* efficacy against both peripheral and intranasal VEEV challenge. Initial studies will evaluate prophylactic efficacy by administering the antiviral compound pre-challenge; inhibitors that shows good prophylactic efficacy will then be tested for therapeutic efficacy. Since highly potent combination therapies targeting different aspects of the viral life cycle are likely to show improved efficacy and limit the development of drug resistance, we also will test whether combinations of small molecule inhibitors and/or Nuc analogs will show improved *in vivo* efficacy against acute VEEV, or acute and chronic CHIKV infection. Lastly, since alphavirus disease is caused, in part, by virus-induced inflammatory immune pathology, we will evaluate the therapeutic potential of combined antiviral and anti-inflammatory therapy to block both virus replication and virus-induced immune pathology.

Aim 3A. *In vivo* Evaluation of Lead Compounds for Efficacy Against alphaviruses.

1. CHIKV Efficacy Testing. ***(a) Protection against acute infection as prophylaxis.*** We will use our established adult C57BL/6 mouse model of CHIKV joint swelling and arthritis [41-44] to evaluate inhibitors for *in vivo* efficacy. By Year 2, lead inhibitors (e.g., **EIDD-1931,** with 36-56% bioavailability in mice) will initially be tested as prophylaxis for the ability to limit CHIKV replication and disease (**Fig 12**). Groups of 10 male and 10 female mice will be either sham-treated or treated with the inhibitor (dose, route and dosing schedule determined by PK studies in Aim 1) 12 h prior to subcutaneous inoculation (sc) in the rear foot with 10^3 PFU CHIKV. Half of the mice (5 males/5 females) from each treatment group will be sacrificed at 72 hpi and viral loads in the inoculated foot, draining lymph node, skeletal muscle, serum, spleen, and distal ankle/joints, will be quantified by plaque assays and quantitative RT-PCR[26, 38, 39]. The remaining mice will be monitored for CHIKV-induced foot/ankle swelling using calipers[44] through day 10 at which time they will be sacrificed, perfused with paraformaldehyde, and H&E stained limbs will be assessed for arthritis, myositis, tenosynovitis, and edema using our established scoring system[42, 43]. Statistical significance of 1) Viral tissue burdens and 2) Foot/ankle swelling between inhibitor-treated and untreated mice will be calculated using a mixed effects model to determine differences in efficacy between different treatment types. Kaplan-Meier method or parametric time to failure models (e.g., exponential or Weibull) will be used to compare time to event (e.g., peak viremia and clearance). Serum, joint and muscle levels of inhibitor will be determined in order to correlate compound concentration and clinical outcomes. ***(b) Therapeutic activity against acute infection and disease.*** Inhibitors found to significantly reduce viral burdens or virus-induced swelling when administered as prophylaxis will be assessed for therapeutic efficacy by initiating administration at 12 and 24 hpi using the same assay and analysis protocol outlined for prophylactic treatment. ***(c) Efficacy in highly susceptible mice.*** Inhibitors found to effectively block virus replication or disease as prophylaxis or therapy also will be investigated for their efficacy in mice deficient in type I IFN responses that are extremely sensitive to CHIKV-induced mortality [39, 41, 45]. *Ifnar1*$^{-/-}$ C57BL/6 mice (JAX mice) will be treated either prophylactically or therapeutically and infected as described above. Since *Ifnar1*$^{-/-}$ mice succumb to low dose CHIKV infection[41, 45], we will use protection from viremia and mortality as readouts of antiviral efficacy. Survival will be analyzed by the Kaplan-Meier method. ***(d) Therapeutic activity against chronic infection and disease.*** Inhibitors showing therapeutic efficacy against acute CHIKV infection will be evaluated for the ability to prevent or mitigate persistent CHIKV replication and inflammation. Studies in humans[22, 46], as well as mouse and NHP models[47, 48], suggest that CHIKV can persistently replicate in joint-associated tissues and cause chronic virus-induced synovitis (**Fig 13**). Therefore, we will test whether inhibitors are capable of preventing or mitigating persistent viral replication and reducing the severity of chronic CHIKV disease. C57BL/6 mice exhibit persistent CHIKV RNA replication in joint tissues that is detectable for several months[30]. We will treat groups of 15 male and 15 female mice during the acute phase (3 dpi) or chronic phase (28 dpi) with candidate inhibitors (or sham control) and levels of viral RNA within the joint tissues will be quantified at 28 dpi (for acute phase treatments) or 3, 7, and 14 days post drug treatment (chronic phase treatment). If we see a reduction in persistent viral RNA levels, we will conduct additional studies to evaluate effects on chronic synovitis and tenosynovitis. ***(e) Resistance.*** We will determine whether the virus develops resistance to drug treatment during acute or chronic infection. If we observe evidence that viruses are escaping treatment, we will use our established deep sequencing approaches to identify associated mutations and these will be re-introduced into our CHIKV cDNA clone to determine whether they confer drug resistance either individually or in combination. Mutations that confer resistance will be further characterized for their effects on viral fitness both *in vitro* and *in vivo*, and we will test whether any of our other lead inhibitors with different modes of action will be effective against these escape mutants.

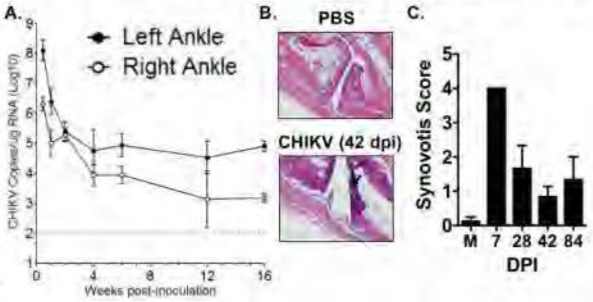


Fig 13. Chronic CHIKV infection and disease in mice. (**A**) WT C57BL/6J mice were inoculated with 10^3 PFU of CHIKV in the left rear footpad and harvested at 3 dpi, and 1, 2, 4, 6, 12, and 16 weeks post-inoculation (n=6-11/time point). CHIKV RNA copy numbers were quantified by absolute qRT-PCR (shown: arithmetic mean ± standard deviation). Dashed line indicates limit of detection. (**B**) Chronic phase (42 dpi) synovitis (black arrow). (**C**) H&E stained sections were scored for the degree of synovitis in ankle joints–scale for percent of tissue affected: 0: absent (0%), 1: minimal (<10%), 2: mild (11-25%), 3: moderate (26-40%), 4: marked (41-60%), and 5: severe (>60%).

(2) VEEV efficacy testing. Epizootic strains of VEEV, such as the Trinidad Donkey (TRD) strain, cause lethal neurologic disease in mice following peripheral or intranasal innoculation[49]. Control of VEEV replication is critical to prevent neurological sequelae and morbidity. Therefore, to test VEEV inhibitors as prophylaxis, we will treat 6-week-old C57BL/6 mice with compounds 12h before footpad inoculation with 10^3 PFU of $\mathrm{VEEV_{TRD}}$. As for CHIKV inhibitors, inhibitor dose, administration frequency, and route will be based on Aim 1 studies. Based on (b) (3) (A), (b) (6) previous experience with VEEV pathogenesis, we will use groups of 20 female mice/treatment group. Negative control mice will receive vehicle only. Sets of 5 mice will be euthanized at 2, 4, and 6 dpi, perfused with PBS and draining lymph node, serum, spleen, brain, and spinal cord assessed for viral load by plaque assay and quantitative RT-PCR, while the last group will be monitored for virus-induced disease (weight loss and neurologic signs). If the antiviral compound shows evidence of efficacy, the study will be repeated in male mice to control for sex difference. Statistical analysis of tissue and serum associated viral titers between the inhibitor treated and control mice will be calculated using Student's t-test. The Kaplan-Meier method will be used to assess the significance of changes in health between sham and compound treated mice. As described above for CHIKV, inhibitors that significantly reduce VEEV burdens and neuroinvasion will be tested for therapeutic efficacy by administration starting at 12 or 24 hpi. Furthermore, if compounds protect from peripheral challenge, we will assess protection against intranasal challenge either prophylactically or therapeutically.

Expected Outcomes and Alternate Strategies. We have established expertise with each of the mouse models used in these efficacy tests, and therefore do not anticipate significant problems interpreting results from these studies. Furthermore, we have established mouse models of infection with a variety of other alphaviruses (RRV, MAYV, ONNV, Una). Compounds that display *in vivo* inhibitory activity against CHIKV and/or VEEV also can be evaluated for *in vivo* protective efficacy against these alphaviruses. Importantly, **EIDD-1931** already shows *in vivo* efficacy (**Fig 12**), so even if issues develop with other lead compounds, we can focus our efficacy testing on **EIDD-1931** and its derivatives. One potential concern with monotherapy is that drug resistant mutants will arise and interfere with treatment efficacy. Therefore, if two or more lead compounds or nucleoside inhibitors show *in vivo* efficacy, we will test (as described above) whether combining compounds results in improved efficacy against acute and chronic CHIKV and/or VEEV infection and disease, as indicated by further decreases in viral replication/disease, the ability to extend the therapeutic window for administration, an overall decrease in the effective dose for one or both inhibitors, and/or a reduction in reversion mutants.

Aim 3B. Will Combining Antivirals with Anti-Inflammatory Drugs Improve Therapeutic Outcomes?

Rationale. Virus-induced inflammation plays a major role in viral pathogenesis. While viral replication is essential role for initiating disease, there is a large body of evidence indicating that an overactive host inflammatory response plays a role in both alphavirus-induced encephalitis[50-52] and acute and chronic arthralgia/arthritis[53]. To ameliorate alphavirus-induced disease, it may be necessary to suppress the inflammatory response. However, the use of anti-inflammatory or immune suppressive drugs may delay viral clearance or even promote viral persistence. Therefore, strategies that inhibit virus replication and dampen inflammation may represent the best approach for treating alphavirus-induced disease. This idea is supported by a recently study by Miner et al., which demonstrated that combining approved Disease Modifying Anti-Rheumatic Drugs (DMARDs), such as CTLA4-IgG, with highly potent anti-CHIKV antibodies resulted in enhanced therapeutic efficacy against CHIKV-induced arthritis compared to either treatment alone[54]. Therefore, as an initial test, we will evaluate whether pairing our lead antiviral compounds with a DMARD such as CTLA4-Ig will enhance our ability to therapeutically treat CHIKV-induced arthritis. These studies will initially focus on combination therapy with **EIDD-1931**, since we know that this nucleoside inhibitor has *in vivo* efficacy against CHIKV. However, if other lead compounds show *in vivo* efficacy against CHIKV, they also will be evaluated using the same methods.

Approach. Prior studies have shown that CTLA4-Ig plus CHIKV antibody treatment at 3 dpi reduced the severity of CHIKV-induced foot swelling and accelerated viral clearance[42, 54]. Therefore, we will use a similar protocol to test whether CTLA4-Ig plus **EIDD-1931** will act synergistically in treating CHIKV disease. As a first test of synergistic effects, sets of 10 (5 male and 5 female) CHIKV infected WT C57BL/6 mice will be treated with either CTLA4-Ig alone (300 µg i.p.), **EIDD-1931** (35 mg/kg, i.p.) alone, or CTLA4-Ig and **EIDD-1931**. A fourth group of CHIKV-infected mice will be sham-treated, while a group of mock infected/sham-treated mice will serve as baseline controls. Mice will be evaluated for foot swelling through day 10 post-infection and then virus-induced inflammatory pathology in joint tissues assessed as in Aim 3A. If CTLA4-Ig+EIDD-1931 treatment shows a synergistic effect, we would repeat the study and measure viral loads and inflammatory cytokine expression at 1, 3, 5, and 7 dpi as described in Aim 3A. If we see a synergistic effect in these initial studies, we would perform additional time of treatment studies to determine the window post disease onset where the combination therapy exhibits therapeutic effects, while also testing whether administration of CTLA4-Ig and EIDD-1931 showed enhanced activity in treating persistent CHIKV disease compared to either treatment alone.

Expected Outcomes and Alternate Strategies. Treatment of patients suffering from alphavirus-induced arthritis is likely to be initiated after the onset of symptoms when host inflammatory responses have already initiated in virus-infected joint tissues. Thus, it may be necessary to use therapies that target both the virus and the inflammatory response[54]. Therefore, it is likely that our highly active leads, such as **EIDD-1931**, when used in combination with CTLA4-Ig will show even greater *in vivo* efficacy. If other leads show good *in vivo* efficacy, they can be tested in combination therapy with DMARDs against CHIKV and other arthritogenic alphaviruses (RRV, MAYV). If combination therapy with CTLA4-Ig does not show improved efficacy over either treatment alone, additional immune modulators can be tested, such as the Jak inhibitor tofacitinib, which has efficacy against CHIKV arthritis in mice[54]. If the combination of CTLA4-IgG or tofacitinib and **EIDD-1931** shows promise, we will assess effects on viral loads during the acute disease. However, if **EIDD-1931** resistance mutants arise, there is the possibility that CTLA4-Ig, or other DMARDs, will delay the clearance of these mutants and potentiate viral persistence. Therefore, we will pay particular attention to this possibility and screen for drug resistant mutants and enhanced viral persistence following combination therapy. Finally, if combined antiviral and anti-inflammatory therapy shows enhanced efficacy against arthritogenic alphaviruses, we will evaluate similar strategies against acute VEEV infection and encephalitis. These studies could evaluate the same DMARDs or other anti-inflammatories such as NSAIDS, which have been shown to be effective against viral encephalitis[55].

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

VERTEBRATE ANIMALS – [redacted] (b) (3) (A), (b) (6)

The use of mice is critical to the experiments described in this application. All animal studies require prior approval by the OHSU animal review committee, and the proposed experiments are an extension of previously approved methods (OHSU IACUCs #0913). The performance site for animals used for antiviral drug efficacy studies will be OHSU (PHS/OLAW Animal Welfare Assurance #- A3304-01). Mice will be used to evaluate antiviral efficacy in mouse models of alphavirus infection and disease.

Description of Procedures:

CHIKV Mouse Model: A total of 600 mice will be used in Years 1-5 of our proposal to assess the efficacy of antiviral compounds in mouse models of CHIKV infection and disease. Equal numbers of Female and Male C57Bl/6 mice (age 4-8 weeks) will be used for these studies. Mice will be anesthetized with isoflurane and inoculated subcutaneously in the footpad with 1,000 pfu CHIKV-SL15649 strain. Blood will be drawn at 3 days post infection to monitor viremia. Swelling in footpad will be monitored by caliper measurements for 7 dpi. Kinetic analyses and tissue harvest will be performed. Under anesthesia, mice will be treated orally or by subcutaneous or intraperitoneal injection with antiviral compounds at concentrations ranging from 5 mg/kg to 25 mg/kg (n=10 mice per group) prophylactically (24 hour pre-infection and then every 24 hpi), or therapeutically (beginning at 8, 12, or 24 hpi; and then every 24 hpi following initial treatment). For kinetics studies, at several time points after infection, the animals will be sacrificed using isoflurane inhalation, and their tissue samples will be harvested and prepared for virologic and immunologic studies. Virologic assays will measure viral burden and tropism at the level of viral RNA, infectious virus production, and viral antigen expression, whereas immunologic experiments will examine whether and how candidate antiviral compounds affect key aspects of severe disease, in particular vascular leakage and cytokine storm. For survival studies, the infected IFNaR-/- mice will be monitored on a daily basis for 30 days; on day 30 post-infection, all mice will be euthanized.

Based upon our similar previous studies in mice, we can detect the following differences between two groups based on n=10 per group, >90% power and 5% significance level for differences between experimental and controls (log10 4.747 vs. 1.77 with standard deviation = 1) using a one-way ANOVA. The power is expected to be higher in actual analyses as we plan to use a mixed effects model, which is more powerful than the t-test or ANOVA. A mixed effects model will be used to measure the change in virus titers (prior to challenge) and determine differences in efficacy between different treatment types. The Kaplan-Meier method or parametric time to failure models (e.g., exponential or Weibull) will be used to compare time to event (e.g., peak viremia, foot pad swelling, and viral clearance).

Justification for Use of Animals:

The goal of this work is the testing of antiviral compound efficacy in a mouse CHIKV infection model. These are sophisticated responses that involve complex interactions between multiple tissue types and cannot be recapitulated *in silico* or *in vitro*. As such it will be necessary to employ animal models to investigate the phenomena we aim to understand. No amount of mathematical modeling can provide the desired information on the effectiveness of the anti-CHIKV therapeutics outlined in this proposal – only *in vivo* studies can provide this information. Mice were selected for the antiviral/infection studies because they represent a tractable *in vivo* model to look at effects on CHIKV replication, dissemination and disease.

Minimization of Pain and Distress:

CHIKV infected mice will be expected to develop clinical disease symptoms (generalized rash, fever, joint pain) as a result of the virus inoculations. Animals will be evaluated for clinical signs of disease on a daily basis by the Division of Comparative Medicine staff. Any animal exhibiting signs of pain or discomfort, including, but not limited to, hunched posture, abnormal behavior, piloerection or decreased food or water consumption, will be brought to the attention of the attending veterinarian and the investigative staff. If pain or discomfort cannot be alleviated with analgesics (buprenorphine and/or carprofen) or other supportive care (soaked chow, subcutaneous fluid therapy), animals will be euthanized. The clinical veterinarian will be responsible for determining if the animal is experiencing any pain or suffering from vaccination or viral infection or from other causes. At the discretion of the veterinarian and in consultation with the principal investigator, animals will receive treatment for treatable conditions, related or unrelated to the experimental protocol.

Discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research. Analgesia, anesthetic, and tranquilizing drugs will be used as determined by our attending veterinarian. Mice will be checked daily by trained animal care personnel.

(b) (3) (A), (b) (6)

[redacted]

Euthanasia:

Mice: Anesthesia will be administered in the form of inhaled isoflurane followed by exsanguination and dissection or cervical dislocation. This method is consistent with the recommendations of the American Veterinary Medical Association.

VERTEBRATE ANIMALS

(b) (3) (A), (b) (6) animal use approval number: 00215
Expiration date: 03/20/2020

1. Description of Procedures. The studies outlined in this proposal utilize mouse models of chikungunya virus (CHIKV) infection and disease to evaluate the in vivo efficacy of candidate antiviral compounds following prophylactic or therapeutic administration. We will perform studies in mice of **both sexes** in experiments proposed as part of Specific Aim 3. As part of this analysis, we will compare outcomes of male and female mice to determine whether sex-based differences exist. If this is observed, we will stratify the analysis and increase the experimental numbers to confirm such findings.

For CHIKV infection studies, young animals (4-5 weeks of age) will be used. CHIKV infection of 4-5-week old WT C57BL/6 mice results in high levels of viral replication within joint tissues, biphasic swelling of the foot, and inflammatory arthritis within the joints of the foot and ankle. Persistent infection and inflammation can be observed for weeks to months. In addition, we will use type I interferon receptor deficient mice (*Ifnar1*$^{-/-}$ C57BL/6), which are highly susceptible to CHIKV disease. Mice will predominantly be inoculated with subcutaneously in the left rear foot. This technique is used routinely in the laboratory to administer volumes up to 20 µl without incident.

For antiviral treatments, the dose, route, and dosing schedule will be determined by PK studies performed as part of Specific Aim 1. In general, we anticipate testing up to three doses and two dosing schedules per compound.

Estimated Number of Mice for Experiments in (b) (3) (A), (b) (6)

SPECIFIC AIM 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease.

Aim 3A. Testing Lead Compounds for In Vivo Efficacy Against CHIKV and VEEV.

Studies in WT C57BL/6 mice:

a) Viruses (n = 1)
b) Mouse genotypes (n = 1): WT
c) Time points (n = 8): acute and persistent phases
d) Compounds (n = 5)
e) Doses (n = 3)
f) Dosing schedule (n = 2): prophylaxis or therapy
g) # of mice per time point (n = 6)
Total: 1 x 1 x 8 x 5 x 3 x 2 x 6 = **1,440 WT C57BL/6 mice**

Studies in *Ifnar1*$^{-/-}$ C57BL/6 mice:

a) Viruses (n = 1)
b) Mouse genotypes (n = 1): *Ifnar1*$^{-/-}$
c) Time points (n = 1)
d) Compounds (n = 5)
e) Doses (n = 3)
f) Dosing schedule (n = 2): prophylaxis or therapy
g) # of mice per time point (n = 6)
Total: 1 x 1 x 1 x 5 x 3 x 2 x 6 = **180 *Ifnar1*$^{-/-}$ C57BL/6 mice**

Breeders: 8 cages rotated twice per year: **16 mice x 2 = 32 *Ifnar1*$^{-/-}$ C57BL/6 mice**

Aim 3B. Will Combining Lead Antiviral Compounds with Anti-Inflammatory Drugs Improve Therapeutic Outcomes?

a) Viruses (n = 1)

b) Mouse genotypes (n = 1): WT

c) Time points (n = 8): acute and persistent phases

d) Compounds (n = 3)

e) Doses (n = 3)

f) Dosing schedule (n = 2): acute and persistent phases

g) # of mice per time point (n = 6)

Total: 1 x 1 x 8 x 3 x 3 x 2 x 6 = **864 WT C57BL/6 mice**

In summary, we estimate that the proposed experiments will require 2,516 mice. Animal care, housing, and procedures will be performed in accordance with PHS Policy on Humane Care & Use of Laboratory Animals and all University of Colorado Denver IACUC policies. All laboratory personnel will attend IACUC-approved training sessions, be IACUC-certified, and be enrolled in the Occupational Health program prior to working with animals.

2. Justifications. The goals of the proposed research are to define the *in vivo* efficacy of new antiviral compounds and combination therapies against acute and chronic CHIKV infection and disease. Thus, these goals require an animal model. There are a number of unique features that make the laboratory mouse the best choice for these studies. Broadly, mice are amenable to genetic manipulation, the complete mouse genome of 15 laboratory mouse strains have been sequenced, and the number of available mouse models continues to increase. These factors enhance the utility of mice as a research model and the ability of researchers to establish mechanisms. More specifically, our model of CHIKV infection in C57BL/6 mice recapitulates many of the acute and chronic disease features experienced by CHIKV-infected humans. Finally, interactions between CHIKV, host tissues, and host immune responses play critical roles in the outcome of infection and the efficacy of therapeutics, and these interactions cannot be fully replicated in tissue culture. Therefore, it is essential that these studies utilize a model that allows for the assessment of disease. There is no substitute for animal models as a way to identify protection against infection and/or disease. In summary, the features of the laboratory mouse as a research model discussed above make it the ideal species for the studies outlined in this application.

3. Minimization of Pain and Distress. Veterinary care will be provided by trained veterinarians on staff at the (b) (3) (A), (b) (6). Monitoring of both breeder and experimental mice will be performed by laboratory research personnel and BSL3-certified animal care personnel. Veterinary staff will communicate directly with laboratory personnel or indicate concerns on specific animal housing cages throughout the experimental protocol. Infection of mice with CHIKV is a potential source of pain or distress. Following viral infection, animals will be observed daily by laboratory and animal care personnel. Any animal exhibiting an inability to eat or drink adequately, a loss of weight in excess of 15% of starting body weight, or markedly reduced response to stimuli will be removed from the study and immediately euthanized.

4. Euthanasia. Euthanasia will be administered by CO_2 gas or isofluorane overdose followed by cervical dislocation or thoracotomy. These methods of euthanasia are consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

VERTEBRATE ANIMALS

1. Description of Procedures. The studies outlined in this proposal utilize mouse models of Venezuelan equine encephalitis virus (VEEV) infection and disease to evaluate the in vivo efficacy of candidate antiviral compounds following prophylactic or therapeutic administration. We will perform studies in mice of **both sexes** in experiments proposed as part of Specific Aim 3. As part of this analysis, we will compare outcomes of male and female mice to determine whether sex-based differences exist. If this is observed, we will stratify the analysis and increase the experimental numbers to confirm such findings.

For VEEV studies, young animals (6 weeks of age) will be used. Wild type VEEV strains result in high levels of viral replication in the periphery of immunocompetent mice (e.g. C57Bl/6), with subsequent viral spread to the central nervous system where the virus will cause neurologic disease. Mice will predominantly be inoculated with subcutaneously in the left rear foot. This technique is used routinely in the laboratory to administer volumes up to 20 μl without incident. If compounds show good prophylactic or therapeutic activity, we will also test them for their ability to protect from intranasal challenge using a 20 ul inoculation containing 10^3 pfu.

For antiviral treatments, the dose, route, and dosing schedule will be determined by PK studies performed as part of Specific Aim 1. In general, we anticipate testing up to three doses and two dosing schedules per compound.

All studies will be performed in a Select Agent BSL3 laboratory that is equipped to handle 180 mouse caged under the direction of (b) (3) (A), (b) (6)

Estimated Number of Mice for Experiments in (b) (3) (A), (b) (6)

SPECIFIC AIM 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease.

Aim 3A. Testing Lead Compounds for In Vivo Efficacy Against VEEV.

Studies in WT C57BL/6 mice (Peripheral Inoculation)

a) Viruses (n = 1)
b) Mouse genotypes (n = 1): WT
c) Time points (n = 4):
d) Compounds (n = 5)
e) Compound Concentrations (n = 3)
f) Dosing schedule (n = 3): prophylaxis or 2 different therapeutic time points
g) # of mice per time point (n = 5)
h) # of sexes (male and female)

Total: 1 x 1 x 4 x 5 x 3 x 3 x 5 x 2 = **1,800 WT C57BL/6 mice**

Up to 3 compounds will be tested for efficacy against intranasal VEEV challenge using optimal dose from the peripheral challenge studies. The other numbers (mouse group size, sex, time points, dosing schedule,…)from the peripheral inoculation studies will stay the same.

Total: 1 x 1 x 4 x 3 x 1 x 3 x 5 x 2 = **360 WT C57Bl/6 mice**

In summary, we estimate that the proposed experiments to test Lead compounds against VEEV will require **2160** mice. Animal care, housing, and procedures will be performed in accordance with PHS Policy on Humane Care & Use of Laboratory Animals and all University of North Carolina at Chapel Hill IACUC policies. All laboratory personnel will attend IACUC-approved training sessions, be IACUC-certified, and be enrolled in the Occupational Health program prior to working with animals.

2. Justifications. The goals of the proposed research are to define the *in vivo* efficacy of new antiviral compounds and combination therapies against VEEV infection and disease. Thus, these goals require an animal model. There are a number of unique features that make the laboratory mouse the best choice for these studies. Broadly, mice are amenable to genetic manipulation, the complete mouse genome of 15 laboratory mouse strains have been sequenced, and the number of available mouse models continues to increase. These factors enhance the utility of mice as a research model and the ability of researchers to establish mechanisms. More specifically, mice are highly susceptible to peripheral and intranasal VEEV infection and represent an accepted model for studying VEEV disease pathogenesis. Finally, interactions between VEEV, host tissues, and host immune responses play critical roles in the outcome of infection and the efficacy of therapeutics, and these interactions cannot be fully replicated in tissue culture. Therefore, it is essential that these studies utilize a model that allows for the assessment of disease. There is no substitute for animal models as a way to identify protection against infection and/or disease. In summary, the features of the laboratory mouse as a research model discussed above make it the ideal species for the studies outlined in this application.

3. Minimization of Pain and Distress. Veterinary care will be provided by trained veterinarians on staff at the (b) (3) (A), (b) (6). Monitoring of experimental mice will be performed by laboratory research personnel and BSL3-certified animal care personnel. Veterinary staff will communicate directly with laboratory personnel or indicate concerns on specific animal housing cages throughout the experimental protocol. Infection of mice with VEEV is a potential source of pain or distress. Following viral infection, animals will be observed daily by laboratory and animal care personnel. Any animal exhibiting an inability to eat or drink adequately, a loss of weight in excess of 20% of starting body weight, or markedly reduced response to stimuli will be removed from the study and immediately euthanized.

4. Euthanasia. Euthanasia will be administered by CO_2 gas or isofluorane overdose followed by cervical dislocation or thoracotomy. These methods of euthanasia are consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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RESOURCE SHARING PLAN

Data sharing: Oregon Health and Science University, University of Carolina at Chapel Hill, and University of Colorado at Denver are committed to the open and timely dissemination of research outcomes. Investigators in this proposed program recognize that promising methods, technologies, data and insights may arise during the course of their research. All investigators are aware of and agree to abide by the principles for sharing research resources, as described by NIH in “Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Programs." The data generated in this grant will be presented at national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. With regards to sequencing data, raw and processed sequencing datasets will be submitted to appropriate public databases (i.e., NCBI SRA and miRBase) following publications.

Plan for sharing model organisms

Through these studies we will be creating a number of recombinant viruses that we anticipate will exhibit unique phenotypes *in vivo*. These recombinant viruses may be useful to other investigators who read our publications or hear about this information at scientific meetings. During our studies, we also anticipate the generation of reagents to study alphavirus replication including but not limited to antibodies, recombinant proteins, and expression vectors. If requests for these recombinant viruses and other reagents are received, we plan to provide these to the investigators after successful completion and approval of material transfer agreements.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Commercially available primary cells and cell lines

Our project will use several primary cell types and cell lines that are available commercially: primary fibroblasts (such as NHDF), Vero cells and C6/36 cells. To ascertain the identity of these cell types and lines, we will purchase them from reliable commercial sources (such as ATCC) before starting the work on the project. Culture medium will be purchased from Corning CellGro and fetal bovine serum from HyClone, both of which are available through Fisher. The cells will be routinely checked for mycoplasma contamination using the MycoAlert detection kit available from Lonza.

Chemicals and biologics (antibodies, cytokine kits, and other biologics)

All chemicals and molecular biology reagents will be purchased from reliable commercial sources. Reagents that do not perform up to standards, based on assays performed in the laboratory, will be replaced. All reagents will be disposed of, once past their expiration date. In addition to data, record keeping will include the vendor information, catalog and lot number for all key biological and chemical resources used in the experiments. This information will be included in the Materials and Methods section of all publications.

Alphavirus RNA detection

Primers/probe combinations for qRT-PCR assays will be purchased and evaluated by conventional PCR to demonstrate that specificity by sequencing the amplicon. RNAScope probes directed against specific alphaviruses will be purchased from Advanced Cell Diagnostics, Inc., which validates specificity and activity of each probe.

Alphavirus strains

CHIKV (strain: OPY-1) was obtained from (b) (3) (A), (b) (6); ONNV, MAYV and RRV were obtained from (b) (3) (A), (b) (6) (b) (3) (A), (b) (6); and additional alphavirus strains will be obtained from (b) (3) (A), (b) (6). We sequence our viral stocks, and any new stocks made from the original virus will be sequenced to ensure sequence stability for reproducibility.

Antibodies and cytokine kits

All primary and secondary antibodies and flow cytometry reagents will be purchased from reputable commercial vendors. We have validated these reagents previously for our studies. Newly acquired antibodies will be tested for efficacy and specificity prior to use. Testing will include western blotting to assess signal to noise ratios, and the immunogen will be included to demonstrate the elimination of specific staining. Controls for immunohistochemistry will also include comparison of positive staining to staining with an irrelevant antibody (negative control) of the same class as the primary antibody in use. Secondary antibodies will be tested in the absence of primary antibody, and only secondary antibodies without signal will be used for such experiments. Antibodies will be titrated to determine antibody dilutions that provide the best efficacy, and specificity Luminex cytokine assays will be purchased from ThermoFisher.